

# An inventory of available laboratory diagnostic tests for selected pathogens along the pig value chain in Vietnam

*Nguyen Tien Thanh<sup>1</sup>, Nguyen Thi Lan Anh<sup>1</sup>, Vu Thi Kim Hue<sup>1</sup>, and Luu Quoc Toan<sup>2</sup>*

*<sup>1</sup>National Institute of Veterinary Research, Vietnam*

*<sup>2</sup>Hanoi School of Public Health, Vietnam*

[www.livestockfish.cgiar.org](http://www.livestockfish.cgiar.org)




September 2014

CGIAR is a global partnership that unites organizations engaged in research for a food secure future. The CGIAR Research Program on Livestock and Fish aims to increase the productivity of small-scale livestock and fish systems in sustainable ways, making meat, milk and fish more available and affordable across the developing world. The Program brings together four CGIAR centres: the International Livestock Research Institute (ILRI) with a mandate on livestock; WorldFish with a mandate on aquaculture; the International Center for Tropical Agriculture (CIAT), which works on forages; and the International Center for Research in the Dry Areas (ICARDA), which works on small ruminants. <http://livestockfish.cgiar.org>

© 2014



This publication is licensed for use under the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported Licence. To view this licence, visit <http://creativecommons.org/licenses/by-nc-sa/3.0/>. Unless otherwise noted, you are free to copy, duplicate, or reproduce and distribute, display, or transmit any part of this publication or portions thereof without permission, and to make translations, adaptations, or other derivative works under the following conditions:

-  **ATTRIBUTION.** The work must be attributed, but not in any way that suggests endorsement by the publisher or the author(s).
-  **NON-COMMERCIAL.** This work may not be used for commercial purposes.
-  **SHARE ALIKE.** If this work is altered, transformed, or built upon, the resulting work must be distributed only under the same or similar license to this one.

ilri.org  
better lives through livestock  
ILRI is a member of the CGIAR Consortium

Box 30709, Nairobi 00100, Kenya  
Phone: +254 20 422 3000  
Fax: +254 20 422 3001  
Email: [ILRI-Kenya@cgiar.org](mailto:ILRI-Kenya@cgiar.org)

Box 5689, Addis Ababa, Ethiopia  
Phone: +251 11 617 2000  
Fax: +251 11 617 2001  
Email: [ILRI-Ethiopia@cgiar.org](mailto:ILRI-Ethiopia@cgiar.org)

# Contents

Abbreviations and acronyms .....	1
Summary .....	2
Introduction .....	3
Objectives .....	3
Methodology .....	4
Results.....	6
General information on interviewees and organizations/institutions	6
Public and private diagnostic laboratories	6
Reference status of organization	6
Contribution to policy development or implementation	7
Functions of each institution/organization	8
Inventory of tests used .....	9
Diagnostic tests for viral pig diseases	9
Tests for PRRS	10
Tests for CSF	11
Tests for FMD	13
Tests for swine flu	14
Tests for <i>B. suis</i>	14
Diagnostic tests for bacterial pig diseases	15
Tests for E.coli septicemia	16
Tests for Pasteurellosis	17
Tests for Salmonella spp.	17
Diagnostic tests for parasitic pig diseases	18
<i>Tests for Cysticercus cellulosae</i>	19
<i>Tests for Trichinella spiralis</i>	20
Tests for fish-borne zoonotic trematodes	20
Vaccines used in the prevention of some pig diseases in Vietnam	21
Limitations of some selected diagnostic tests in detecting pig diseases.....	22
Potential diagnostic opportunities to be developed .....	23
Conclusions .....	24
Annex 1: Institutions engaged in detecting major pig diseases and the number of tests conducted per year.....	25
Annex 2: Senior advisers consulted in this inventory .....	26
Annex 3: Respondents interviewed .....	27
Annex 4: Questionnaire and guidelines .....	28
Annex 5: References .....	36

# Abbreviations and acronyms

HSPH	Hanoi School of Public Health
HUA	Hanoi University of Agriculture
DAH	Department of Animal Health
NIVR	National Institute of Veterinary Research
HMU	Hanoi Medical University
Sub-DAH-HCM	Sub- Department of Animal Health of HCM city
DVS	District Veterinary Station
RTD jsc	Rural Technology Development (joint stock company)
CP jsc	Charoen Pokphand Group (joint stock company)
CGIAR	Consultative Group on International Agricultural Research
PRRS	porcine reproductive and respiratory syndrome
CSF	classical swine fever
FMD	foot and mouth disease
FBD	food-borne disease
NIN	National Institute of Nutrition
RAHO	Regional Animal Health Office
NIFC	National Institute of Food Control
NLU	Nong Lam University
NCVD	National Centre for Veterinary Diagnostics
Ab	antibody
Ag	antigen
Se	sensitivity
Sp	specificity
PED	porcine epidemic diarrhea
ACIAR	Australian Centre for International Agricultural Research
ISO	International Organization for Standardization
IPMA	immuno peroxidase monolayer assay
ELISA	enzyme-linked immunosorbent assay
PCR	polymerase chain reaction
RT-PCR	real-time polymerase chain reaction
PCR-RFLP	polymerase chain reaction- restriction fragment length polymorphism
Ab-ELISA	antibody enzyme-linked immunosorbent assay
Ag-ELISA	antigen enzyme-linked immunosorbent assay
PVC2	porcine circovirus type 2
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
VND	Vietnam dong
TCVN	Vietnam standard
B. suis	Brucella suis
C. cellulosa	Cysticercus cellulosa
C. tenuicollis	Cysticercus tenuicollis
T. spiralis	Trichinella spiralis

# Summary

Pigs account for a significant share of output from the livestock sector in Vietnam, with 26.98 million pigs recorded in April 2013, 1.08% higher than the previous year's output. Pork is the dominant meat consumed by Vietnamese consumers. In 2012, 1.94 million tons of pork were consumed. Production has slowly increased to catch up with demand, but there were constraints related to animal health. To address such constraints, an understanding of disease drivers and the underlying factors is critical. Just as essential is putting in place surveillance and control measures, supported by appropriate diagnostic tools. . This study aims to provide an inventory of diagnostic tools available in Vietnam to identify key pathogens along the pig value chain in the country.

Interviews were conducted and named respondents were 12 staff members from government institutions/organizations and three from a private company. The questionnaire asked about respondents' demographics, vaccine use, available diagnostic tests, and advantages/disadvantages of tests used to detect pig diseases in Vietnam. Information were recorded, coded, and analyzed in Microsoft Excel 2013. The results showed that 14 diagnostic tests were being used to detect pig diseases. ELISA and PCR were commonly used to detect viral and parasitic pig diseases. The isolation test was mainly applied to detect bacterial pig diseases. Most of the protocols were based on tests already established abroad, except for a few that were developed in Vietnam.

Advantages of each test were described in details - e.g., direct detection of bacteria or virus by the isolation test, the high accuracy of PCR test, or the simplicity and accuracy of ELISA. Disadvantages were also reported - e.g., cross-reaction when using Ab-ELISA to detect PRRS, CSF, *Trichinellosis*; tests that are time-consuming (ELISA and PCR), expensive, need standard virus or bacteria for culture (isolation test), require equipment and expertise of technicians; currently available tests that could not be applied under field condition, slaughterhouses, or in the market. Therefore, the development of rapid test kits (e.g., those for *Salmonellosis*, *Cysticercosis*, *Trichinellosis*) that can give quick and accurate results, that are cheaper, and that can be easily used by lab technicians, farmers, or consumers is critical in preventing and controlling zoonotic pig diseases in Vietnam.

Key words: pig/pork, diagnostic tests, pathogens, zoonotic diseases, Vietnam

# Introduction

The International Livestock Research Institute (ILRI) and its CGIAR partners are implementing a global program on Livestock and Fish in eight value chains across different countries and continents. The overall goal of the CGIAR Research Program is to sustainably increase the productivity of small-scale livestock and fish systems to increase the availability and affordability of animal-sourced food for poor consumers, and in so doing, reduce poverty through greater participation by the poor along the value chains for animal-sourced food. One of the value chains of interest is the pig value chain in Vietnam.

Pigs account for a significant share of output from the livestock sector in Vietnam. The Ministry of Agriculture and Rural Development reported that, in April 2013, the number of pigs in Vietnam reached 26.98 million, 1.08% higher than the previous year's figures. Pork is the dominant meat consumed in the country. The amount of meat consumption in 2012 was about 1.936,2 thousand (1,936,200?) tons (Chien 2013). Production has slowly increased to catch up with demand, however, there were various challenges, including those related to animal health. To address such constraints, surveillance and control measures need to be in place, supported by appropriate diagnostic tools, besides achieving a greater understanding of disease drivers and the underlying factors. In spite of the existence of a wide range of diagnostic tools in the market, there is limited information on the specific tools and tests applied by national institutions and the private sector. Also wanting are the scope of use, availability, and costs. ILRI's Bioscience Unit has developed a set of promising, cheap, and easy-to-use diagnostic tools, but demand for such tools has not yet been explored in many countries, including Vietnam.

This study aims to provide an inventory of available diagnostic tools for selected key pathogens along the pig value chain in Vietnam. It will analyze these tools to identify gaps and needs in order to develop or adapt their use in the local context. Apart from this, output from this work is expected to contribute to the development of a broader laboratory diagnostic scheme and here, ILRI's Bioscience Unit could play a role in terms of sharing knowledge and designing potential novel diagnostic tools that may be used in the field or the laboratory.

## Objectives

- To provide an inventory of available diagnostic tools for selected key pathogens along the pig value chain
- To appraise the limitations of these tools in terms of efficacy, sensitivity, specificity, ease of use, cost, and availability

# Methodology

## Tool development

The inventory study was conducted in December 2013. Guidelines for key informant interviews were developed and tested, including ways to obtain information on i) the role of institutions/organizations involved, ii) pig vaccinations applied or produced as they relate to the selected pathogen, iii) specific tests used to detect a certain pathogen, iv) known diagnostic challenges for each test that should be specifically improved or targeted for development, and v) potential opportunities for application of novel techniques (Annex 1).

## Identification of key informants

There is no specific laboratory that makes a diagnosis of pig diseases in Vietnam. The laboratories capable of diagnosing swine diseases are the seven regional animal health office laboratories (RAHO - DAH) and the National Center for Veterinary Diagnosis (NCVD) – DAH. The National Institute of Veterinary Research (NIVR) has experience on diagnosing diseases. Universities such as the Hanoi University of Agriculture (HUA), Nong Lam University (NLU), and Hue University mostly involve student practitioners. The Sub-DAH personnel screen pig diseases in the field and send all samples to RAHO, NCVD, and NIVR.

Key informants who have experience in lab and diagnostic work and who have animal health and public health background were selected from various institutions and organizations. These institutions and organizations consisted of existing partners in previous or ongoing ILRI projects (e.g., Faculty of Veterinary Medicine – HUA, NIVR, and Hanoi School of Public Health - HSPH). Other key informants were selected based on their importance as a potential resource person and collaboration with our networks. Key informants from the private sector were also included: these were key drug distributors and integrated pig producers. Respondents were identified through the help of senior advisers from NIVR, HUA, –NCVD, and HSPH. Participants were in various positions. Most of them were in middle/upper management in an organization. They had a good understanding of pig production and pig health as well as extensive lab experience. In total, 19 persons (Annex 3) were identified and invited to an interview. International colleagues (CIRAD, FAO, WHO) in Vietnam were identified but the pre-tests revealed that their work and background are way too far from diagnostic tools.

## Interviews

Respondents (Annex 2) were contacted through phone calls or email. The interviews were conducted in places where both respondent and interviewer felt most comfortable. The interview took about 50-70 minutes. All information were recorded in answer sheets (Annex 4).

## Pathogen identification

The diagnostic tests included in this inventory mainly relied on pathogens identified in an ongoing ACIAR-funded project targeting pig value chains in two regions of Vietnam. Table 1 provides an overview of the pig pathogens incorporated in this inventory.

Table 1: Main pig pathogens in Vietnam

No.	Pig diseases		Food-borne diseases/zoonoses	
	Viral/bacteria	Parasitic/others	Viral/bacteria	Parasitic/others
1	PRRS*	Roundworms	Salmonella spp.*	Cysticercus cellulosae*
2	CSF*	Mange	Campylobacter spp.	Trichinella spiralis*
3	FMD*		Streptococcus suis*	
4	E. coli septicemia*		Leptospirosis*	
5	Erysipelas*		Japanese encephalitis	
6	Pasteurellosis*		Brucella suis	
7	Paratyphoid suum*		Swine flu (H1N1)*	
8	Mycoplasma			

\* Listed in the animal health review by the Faculty of Veterinary Medicine - HUA (ACIAR project).

## Data entry and analyses

Data from questionnaires were recorded in an Excel 2013 spreadsheet with diseases treated separately. These included data about the interviewees (organization, job, experience); vaccine (type, own produced, challenges); diagnostic tests (steps, sensitivity, specificity). Each diagnostic test was given a code; sensitivity was coded as high (>80%); medium (50-80%); and low, otherwise, real figures were recorded whenever available. The same was true for specificity and efficacy. The data were analyzed using the Excel tool.



# Results

## General information on interviewees and organizations/institutions

### Public and private diagnostic laboratories

Among the 19 interviewees (11 males and 8 females), 15 work for the government and the rest are employed by the private sector. Fifteen institutions/organizations were involved in this survey (Table 2).

Table 2: Public and private diagnostic laboratories included in this inventory

Type of organization	Location		
Government	Hanoi	Ho Chi Minh	Hai Phong
	- National Institute of Veterinary Research (NIVR)**	-Regional Animal Health Office VI (RAHO 6)	- RAHO 2
	- National Centre for Veterinary Diagnostics (NCVD)*	-Nong Lam University (NLU)	
	- Veterinary Station of Thach That district (DVS-TT)	-Sub-DAH-HCM	
	- National Institute of Nutrition (NIN)	- Pasteur Institute of Ho Chi Minh City (PI-HCM)	
	- National Institute for Food Control (NIFC)		
	- Hanoi University of Agriculture (HUA)		
Private	- Hanoi Medical University (HMU)		
	- Rural Technology Development Jsc (RTD Jsc.)	VIFAVET	
	- CP Vietnam*		

\*The organization had two interviewees; \*\* the organization had three interviewees.

Twelve of the 15 institutions/organizations are run by the government: 3 national institutes and 8 from NCVD (1), the veterinary station of the district (1), universities, (3) sub-DAH (1), PI-HCM (1), and RAHO (2). Most of the interviewees are veterinarians, except for three medical doctors from NIN, NIFC, and HMU; one is from the biomedical and pharmacology field. Average age of interviewees was 39 (range, 28–59); their laboratory experience ranged from 1 to 27 years.

### Reference status of organization

Animal diseases, public health, and food safety are the key activities of the interviewees' organizations. Most of the institutions/organizations have laboratories that conduct diagnostic tests and have certifications that they meet the international (9/19), national (7/19) or local standards (2/19). The exception was the district veterinary station that only has a refrigerator to preserve vaccines; they had to send samples to DAH for diagnosis (Table 3).

Table 3: Laboratories' reference status

Organization	Lab's reference status		
	International standard	National standard	Local standard
NIVR		√	
NCVD	√	√	
DVS-TT			
NIN	√		
NIFC	√		
HUA	√		
HMU	√		
Sub-DAH-HCM		√	
RAHO 2		√	
RAHO 6			√
NLU		√	
PI-HCM	√		
RTD	√		
CP Vietnam	√		
VIFAVET		√	

Among the 15 institutions/organizations, 8 institutions/organizations reported that their laboratories satisfy international standards. NCVD, RTD, CP Jsc, NIFC, NIN, and HUA received the ISO 17025 recognition (Laboratory Assurance); PI-HCM received ISO 15189 and ISO 17025. The rest were following ISO but had to be modified. Apart from that, six laboratories had reference status as meeting the national standard and one satisfying the local standard.

#### .1.2. Roles of laboratory in regulation development/implementation

Of the 15 institutions/organizations, 12 were involved in regulation development or implementation. The functions reported are listed in Table 4.

Table 4: Laboratories involved in regulation development/implementation

Organization	Yes	Detail	No
NIVR	✓	Ideas for regulation development	
NCVD	✓	Consult and contribute ideas to regulation development	
DVS-TT	✓	Instruction and monitoring of law enforcement	
NIN	✓	Vietnamese standards for food	
NIFC	✓	Draft national standard on food safety	
PI-HCM	✓	Research, outbreak control, produce vaccine, services	
HUA	✓	Referee function, technical confirmation to help declare epidemic disease	
HMU	✓	Consult, prepare instructions and regulations	
Sub-DAH-HCM			✓
RAHO 2	✓	Participating in regulation development through workshops or conferences held by DAH or making a proposal to DAH	
RAHO 6	✓		
NLU		Ideas for regulation development	
RTD			✓
CP Vietnam	✓	Reference function between labs with organizations GD (Holland) and IFM (Australia)	
VIFAVET			✓

#### Contribution to policy development or implementation

Ten institutions/organizations were involved in policy development/implementation. The activities included providing ideas, giving recommendations to policymakers (e.g., Codex); developing a vaccine; providing consultancies, analyzing samples, training and research, policy development in outbreak investigation, epidemic control, and vaccination.

## Functions of each institution/organization

The functions of each organization are listed in Table 5.

Table 5: Functions of each organization

Organization		Function		
		Diagnosis	Prevention	Research and diagnosis
Government	NIVR	√	√	√
	NCVD	√	√	
	DVS-TT		√	
	NIN	√		
	NIFC	√		
	PI-HCM	√	√	√
	HUA	√	√	√
	HMU	√	√	
	Sub-DAH-HCM	√		
	RAHO 2	√	√	
	RAHO 6	√	√	
	NLU	√		
Private company	RTD Jsc.			√
	CP	√	√	
	VIFAVET	√		

The main function of most laboratories was to diagnose diseases (14/15), except for DVS-TT, which did not have a laboratory and diagnosed pig diseases mostly based on symptoms and technical experience; for this lab, samples must be sent to NCVD for diagnostic tests if needed. Only 9 labs reported involvement in the prevention of pig diseases, either directly by participating in pig vaccination (CP, RAHO2, HMU, and DVS-TT) or indirectly by developing vaccines (NIVR, HUA, PI-HCM). In addition, a combination of research and diagnosis was done by NIVR, HUA, PI-HCM, and RTD Jsc. Apart from the institutions/organizations of government, private companies often conduct diagnostic tests in pigs for their own farms or for farmers as customer service if piglets, medicine, or vaccines were bought from them.

As to pathogens, most of the institutions/organizations focus on detecting viral, bacterial, and parasitic pathogens (12/15) but with more emphasis on the first two, especially on zoonotic diseases or diseases that cause outbreaks and economic losses for the pig industry.

The pig diseases identified in this survey are listed in Table 1, but some diseases that are less frequently tested in laboratories or where there were few information obtained are not shown (e.g., PED, erysipelas, mange, or campylobacteriosis).

In general, to detect diseases in pigs, 14 diagnostic methods were used. These were enzyme-linked immunosorbent assay (ELISA); polymerase chain reaction (PCR); culture; virus neutralization test; immunoperoxidase monolayer assay (IPMA); test for antibiotic sensitivity; histopathology; meat inspection; sedimentation or flotation method; digestion method; combination method of sedimentation, flotation, and centrifugation; morphological identification; rapid agglutination test (e.g., rose of Bengal). Tests used to detect virus, bacteria or parasites in pigs are listed below. Apart from the normal tests, ELISA tests (either Ab or Ag detection) and/or PCR (real time [RT]-PCR or PCR-RFLP) were being implemented in 10 of 15 institutions/organizations either from the government or the private sector. The NIN and NIFC did not report about doing PCR and ELISA tests at their labs. No information was obtained about diagnostic tests used at PI-HCM. No information on rapid tests was recorded.

# Inventory of tests used

## Diagnostic tests for viral pig diseases

Table 6: Major viral diseases tested by institution/organization

Institution organization	Major viral diseases				
	PRRS	CSF	FMD	Swine flu	PED
NIVR	√	√	√	√	√
NCVD	√	√	√	√	√
HUA	√	√	√		
HMU			√		
PI-HCM	√	√	√	√	
RAHO2	√	√	√	√	√
RAHO6	√	√	√	√	
Sub-DAH-HCM	√	√	√	√	
NLU	√	√	√		√
RTD Jsc.	√	√	√		√
CP company	√	√	√	√	
VIFA VET	√	√			

Viral pig diseases were diagnosed in all institutions/organizations, especially PRRS, CSF, and FMD, which were sometimes reported as outbreaks in Vietnam.

In general, five diagnostic tests were conducted to detect viral pig diseases (Table 7).

Table 7: Diagnostic tests used to detect selected viral diseases in pigs (N=11)

Test	Diseases				
	PRRS	CSF	FMD	H1N1	PED
ELISA	√	√	√	√	
RT-PCR	√	√	√	√	√
Isolation	√	√	√	√	
IPMA	√				
Neutralization test	√	√	√		

N: number of interviewees

Data from Table 7 showed that, to detect major viral pig diseases in Vietnam, apart from traditional tests such as isolation, neutralization and IPMA, ELISA and PCR were commonly used. ELISA and PCR tests were conducted in 10 and 9 institutions/organizations, respectively, except for DVS-TT, NIN, NIFC, and HMU (either it was not their lab's function (NIN, NIFC and HMU) or no equipment is available (DVS-TT)). PCR was not conducted by VIFAVET.

Detail information on the tests conducted in each institution/organization are presented below.

## Tests for PRRS

Diagnostic tests to detect PRRS were reported by 11 of 18 interviewees (Table 8).

Table 8: Tests used for detecting PRRS

Organization		Test				
		ELISA	RT-PCR	Isolation	IPMA	Neutralization
Government	NIVR		✓	✓	✓	✓
	NCVD	✓	✓	✓		
	HUA		✓	✓		
	PI-HCM					
	Sub-DAH-HCM	✓	✓			
	RAHO 2	✓	✓			
	RAHO 6	✓	✓			
	NLU	✓	✓			
	RTD Jsc.	✓	✓			
	CP company	✓	✓			
Private	VIFA VET	✓				

Twelve interviewees mentioned five diagnostic tests used to detect PRRS in pigs. Of these, ELISA and RT-PCR were conducted at the laboratories of 9 and 10 interviewees, respectively, both government institutions/organizations and private companies. No information about diagnostic tests used at PI-HCM were given. Detailed information on each test used for detecting PRRS are presented in Table 9.

Table 9: Detailed information on each test used for detecting PRRS.

Criterion	ELISA	RT-PCR	Isolation	IPMA	Neutralization test
No. of tests/year/lab	100 -18,500	20 -12,850	10 -1,000	500	60
Sensitivity (agree/interviewees)	High (4/9); (5/9)*	High (5/10); (5/10)*	High (2/3)	High (1/1)	High (1/1)
Specificity (agree/interviewees)	High (3/9); Medium (1/9); (5/9)*	High (5/10); (5/10)*	High (3/3)	High (1/1)	High (1/1)
Cost (x1000 VND) (who pays)	100-150 (company, farmers, projects)	600 (company, farmers, project)	100 Depends on project	TCVN	TCVN
Availability (agree/interviewees)	Yes	Yes	Yes	Yes	Yes
Feasibility (agree/interviewees) (at central, local, farm level)	Local (6/9); farm (1/9); (2/9)*	farm (2/10); local (6/10); (2/10)*	Central (2/3); farm (1/3)	Central	Central
Routine/request (%) (agree/interviewees)	Both	Request	Both	Request	Request
Efficacy	High (2/9); medium (2/9); (5/9)*	High (6/10); (4/10)*	High (3/3)	High	High
Advantages	Cheap (2/9), simple (1/9), quick (2/9), accurate (1/9), detects antibody (2/9), (2/9)*	Quick, accurate, high level of confidence, detects virus	Cheap, can collect virus, can collect live virus	Detects antibody	Detects antibody
Disadvantages	Cross-reaction when vaccinated Only detects antibody. Cannot differentiate between vaccinated and infected pigs	Expensive (2/10), need to update information on the test	Depends on quality of samples and can be done only if virus is alive	None	None
Origin of test	Other countries	Other countries	Other countries	Other countries	Other countries

\*=Do not know

-Agree/interviewees= number of interviewees who agreed with each point (high, medium, or low) in each criterion for each test out of the total number of interviewees who have used the test

TCVN=follows Vietnam standard

As shown in Table 9, the most frequently used tests were Ab-ELISA (up to 18,500 by CP), followed by RT PCR (up to 12,850 by Sub-DAH-HCM). Virus isolation was done (up to 1,000) by HUA. The reasons for using ELISA and PCR may have something to do with their cheap price and the simplicity of the test procedures. However, four interviewees reported difficulties in differentiating PRRS-infected pigs from vaccinated pigs by using ELISA. In contrast, PCR was conducted in the laboratory of 10 interviewees but there were fewer tests/year. This might be because the test is expensive though accurate results were obtained and there was a high level of confidence when using the test. The neutralization test to detect PRRS was done at NIVR with only a small number of tests per year. All five tests used to detect PRRS in Vietnam originated from other countries.

### Tests for CSF

To detect CSF, diagnostic tests were being conducted in 11 institutions/organizations (Table 10).

Table 10: Diagnostic tests used for detecting CSF

Organization		Test				
		ELISA	RT-PCR	Isolation	Neutralization test	S/N titer
Government	NIVR	√	√		√	
	NCVD		√	√	√	
	HUA	√	√	√		
	PI-HCM					
	Sub-DAH-HCM	√	√			
	RAHO 2	√	√			
	RAHO 6	√	√			
Private	NLU	√	√			
	RTD Jsc.		√			
	CP	√	√			√
	VIFA VET	√				

Table 10 shows PCR and ELISA being conducted in 9 and 8 institutions/organizations, respectively. The three other tests were rarely used. No difference was seen between institutions/organizations belonging to government and to the private sector in their use of ELISA or PCR. No test information was taken from PI-HCM.

Information on diagnostic tests used to detect CSF are given in Table 11.

Table 11: Diagnostic tests to detect CSF

Criterion	ELISA	RT-PCR	Isolation	Neutralization test	S/N titer
No. of tests/year/lab	100 - 7000	20 - 400	100	100	4000
Sensitivity (agree/interviewees)	High (3/9); (6/9)*	High (4/9); (5/9)*	High (1/1)	High(1/1)	(1/1)*
Specificity (agree/interviewees)	High (3/9); (6/9)*	High (4/9); (5/9)*	High (1/1)	High (1/1)	(1/1)*
Cost (x1000VND)	100	700 or TCVN	*	*	70
Availability (agree/interviewees)	Yes (5/9)	Yes (4/9)	Yes	Yes	Yes
Feasibility (at central, local, farm level)	Local (4/9); farms(2/9); (3/)*	Central (3/9); farm (2/9); (4/9)*	Farm	Central	Farm
Routine/request (%) (agree/interviewees)	Different, depends on each lab	Request (5/9); (4/9)*	20/80 (1/1)	0/100	95/5
Efficacy	High (3/9); medium (2/9); (4/9)*	High (4/9); (5/9)*	High(1/1)	High	High
Advantages	Highly accurate (1/9), cheap, simple (1/9); quick, detects Ab (3/9); (4/9)*	Highly accurate (2/9), detects Ag (2/9); (5/9)*	Can obtain live virus	Detects antibody	Can differentiate between Ab (vaccinated vs diseased)
Disadvantages	Expensive (1/9), only detects Ab, cannot differentiate between vaccinated and infected pigs (3/9); No constraints (1/9); (4/9)*	Expensive (2/9), regular samples will be better (1/9), no constraint (1/9), (5/9)*	Need standard virus for culture	None	None
Origin of test	Other countries	Other countries	Other countries	Other countries	Other countries

\*=Do not know

TCVN=follows Vietnam standard

All 12 interviewees reported the conduct of diagnostic tests in their laboratories to detect CSF. ELISA and PCR were reported by nine interviewees while either isolation or neutralization or SN titer test was reported by one interviewee. Many samples were diagnosed using ELISA at NCVD, RAHO6, CP, and VIFAVET because private companies gave free diagnostic tests to farms for the prevention of CSF in pigs. On the other hand, the main function of NCVD was to diagnose diseases and samples were sent from sub-DAH; RAHO's function involves diagnosis and prevention. S/N titer was only implemented at CP Jsc. Many interviewees did not know exactly what the Se and Sp of the tests are. The levels of Se and Sp were determined based on experience. Apart from one respondent who knew the advantages and disadvantages of the tests, all the others had no idea. Most of the tests used originated from other countries. The cost of the test was not exactly calculated by interviewees; sample responses were 'as regulated' or 'farmers pay for it' or 'it depends on the project or supporter.'

## Tests for FMD

In the case of FMD, diagnostic tests were conducted in 11 organizations/institutions. Diagnostic tests used for detecting this disease in each organization are listed in Table 12.

Table 12: Diagnostic tests used for detecting FMD

Organization	Test				
	ELISA	PCR	Isolation	Neutralization test	S/N titer
NIVR		√		√	
NCVD	√	√			
HUA	√	√	√		
HMU		√			
PI-HCM					
Sub-DAH-HCM	√	√			
RAHO 2	√	√			
RAHO 6	√	√			
NLU	√	√			
RTD Jsc.		√			
CP	√	√			√
VIFA VET	√				

To detect FMD, five diagnostic tests were conducted; PCR and ELISA were the ones commonly used. No information about tests used at PI-HCM was obtained.

Table 13: Tests used for detecting FMD

Criterion	ELISA	PCR	Isolation	Neutralization test
No. of tests/year/lab	50-12,924	10-200	20-200	300
Sensitivity (agree/interviewees)	High (4/8); (4/8)*	High (3/8); (5/8)*	High (2/3)	High (1/1)
Specificity (agree/interviewees)	High (2/8); Medium (2/8); (4/8)*	High (3/8); (5/8)*	High (3/3)	High (1/1)
Cost (x1000 VND)	(4/8)*; 185 (1/8); 300 (1/8); as a rule (1/8); high (1/8)	(4/8)*; 500 (1/8); 700 (1/8); high (1/8); farmers pay (1/8)	100 (1/3); depend on project (1/3); (1/3)*	(1/1)*
Availability (agree/interviewees)	Yes (4/8); (4/8)*	Yes (4/8)	Yes (3/3)	Yes (1/1)
Feasibility (at central, local, farm level)	Central (3/8); farm (2/8); (3/8)*	Central (3/8); farm (2/8); (3/8)*	Central (3/3)	Central
Routine/request (%) (agree/interviewees)	Both	Request (2/8); (6/8)*	Mainly per request (2/3)	Routine
Efficacy	High (3/8); (5/8)*	High (2/8); medium (1/8); (5/8)*	High (3/3)	High
Advantage	Detects Ab	Quick (2/8); Se (1/8), detects virus; virus is isolated (1/8); (4/8)*	Have live virus and virus isolated	Detects Ab
Disadvantage	Not high Sp (1/8), expensive (1/8); no constraint (1/8); (5/8)*	Expensive (1/8); cannot differentiate serotypes (1/8); (6/8)*	Expensive, need standard virus for culture	None
Origin of test				

\*=Do not know

ELISA and PCR tests were most frequently used, as reported by eight interviewees. The isolation test was cited by three respondents and one mentioned the neutralization test. The highest number of tests/year was seen in ELISA at Sub-DAH-HCM (12,924 samples) and CP (1,000 samples) and the lowest was observed at RTD Jsc. with only 50 samples. The high cost of ELISA, PCR, and isolation test was reported. PCR and isolation tests allowed direct detection of the virus, in contrast to ELISA and neutralization tests that were used to detect Ab against FMD. In addition, S/N titer was applied to detect FMD at CP with 300 samples/year with no constraint.



## Tests for swine flu

Table 14: Tests used for detecting swine flu

Criterion	ELISA	PCR	Isolation
No. of tests/year	10 - 4000	3 - 100	4000
Sensitivity (agree/interviewees)	High (1/2)	High (1/5); (4/5)*	Medium (1/1)
Specificity (agree/interviewees)	High (1/2)	High (1/5); (4/5)*	High
Cost	*	*	*
Availability (agree/interviewees)	Yes	Yes	Yes
Feasibility	To farm (1/2)	Central (1/5); * (4/5)	Central
Routine/request (%) (agree/interviewees)	0/100	Request	Request
Efficacy	High	High	High
Advantages	Detects Ab	Detects Ag; quick	Detects Ag
Disadvantages	None	None	None
Origin of test	Other countries	Other countries	Other countries

\*=do not know

Diagnostic tests for swine flu were reported by seven interviewees who came from Sub-DAH\_HCM, HMU, RAHO2, RAHO6, PI-HCM, CP, and NIVR. PCR was conducted in the laboratories of five interviewees. Isolation and ELISA tests were used at NIVR with 4,000 samples tested/year.

## Tests for *B. suis*

To detect *B. suis*, a rapid agglutination test was used by RAHO2 and Sub-DAH-HCM. Detailed information on the test is presented in Table 15

Table 15: Tests used for detection of *B. suis*

Criterion	Rapid agglutination test (Rose of Bengal)
No. of tests/year	29
Sensitivity(agree/interviewees)	Low (1/2). (1/2)*
Specificity (agree/interviewees)	(2/2)*
Cost	Farmers pay
Availability (agree/interviewees)	Yes
Feasibility	Local (1/2); (1/2)*
Routine/request (%) (agree/interviewees)	(2/2)*
Efficacy	Medium
Advantages	Rapid, cheap, simple
Disadvantage	Low Se
Origin of test	Other countries

\*=o not know

## Diagnostic tests for bacterial pig diseases

Bacterial pig diseases were reportedly conducted in the laboratories of 13 institutions/organizations (Table 16). Bacterial pig diseases were not conducted by RTD.

Table 16: Major bacterial diseases tested by institutions/organizations.

Institution organization	Selected bacterial diseases				
	E. coli septicemia	Pasteurellosis	Salmonellosis	Streptococcus suis	Mycoplasmosis
NIVR	√	√	√	√	√
NCVD	√	√	√	√	√
HUA	√	√	√	√	√
HMU	√		√	√	√
PI-HCM	√	√	√	√	√
Sub-DAH-HCM	√	√	√		
RAHO2		√	√		√
RAHO6		√			√
NIFC	√		√		
NIN	√		√		
NLU	√	√		√	√
CP company		√			√
VIFA VET	√	√		√	

Data from Table 16 show pigs in Vietnam being examined for five major bacterial diseases (five diseases evaluated at NIVR, NCVD, HUA, PI-HCM and four diseases at NLU and HMU). *E. coli septicemia*, *Pasteurella* spp. were tested in 10 laboratories.

Interviewees mentioned four different diagnostic tests or related techniques to detect bacterial pig diseases (Table 17).

Table 17: Tests used to detect bacterial diseases

Test	E. coli septicemia	Pasteurellosis	Salmonellosis	Campylobacter	Streptococcus suis	Mycoplasmosis	Paratyphoid suis
ELISA	√	√				√	
PCR		√			√	√	
Isolation	√	√	√	√	√	√	√
Test for antibiotic sensitivity		√	√		√		√

Among the reported tests, isolation was mainly used to detect bacterial pig diseases and the test for antibiotic sensitivity was then used.. Information on diagnostic tests used to detect bacterial pig diseases in each institution/organization is shown in Table 18.

Table 18: Diagnostic tests used to detect bacterial pig diseases, by institution/organization

Organization	Test				
	ELISA	PCR	Isolation	Test for antibiotic sensitivity	
Government	NIVR	✓	✓	✓	
	NCVD	✓	✓	✓	✓
	HUA		✓	✓	✓
	HMU		✓	✓	
	PI-HCM				
	Sub-DAH-HCM				
	NIN			✓	
	NIFC			✓	
	RAHO 2	✓		✓	
	NLU		✓	✓	
	CP company	✓	✓	✓	
	VIFAVET			✓	
Private					

### Tests for E.coli septicemia

Table 19: Information on each test used for detecting *E. coli septicemia*

Criterion	ELISA	PCR	Isolation
No. of tests/year/lab	1000	300	150 – 4000
Sensitivity (agree/interviewees)	High (1/1)	High (1/2); (1/2)*	High (4/9); 70% (1/9); (4/9)*
Specificity (agree/interviewees)	High (1/1)	High (1/2); (1/2)*	High (3/9); Low (1/9); 40% (1/9); (4/9)*
Cost (x1000 VND)	50	400	70 (1/9); 150 (1/9); 280 (1/9); 300 (1/9); depend on project (1/9); (4/9)*
Availability (agree/interviewees)	Yes	Yes	Yes
Feasibility	Central and local	Central (1/2); (1/2)*	Central (1/9), local (2/9), farm (2/9); (4/9)*
Routine/request (%) (agree/interviewees)	0/100	0/100	Both
Efficacy	High	High (1/2)	High (4/9); medium (1/9); (4/9)*
Advantages	Detect Ab	High accuracy	Can test (later) for antibiotic sensitivity; live bacteria can be obtained; know antibody due to vaccinated or infected
Disadvantages	None	Equipment expensive	Labor-intensive and needs standard bacteria for culture
Origin of test	Other countries	Other countries	Other countries (NIVR, HUA and NLU); Own produced (VIFAVET); both (CP Jsc)

\*=do not know

The isolation test was mainly used to detect *E. coli septicemia* (9 out of 10 interviewees reporting) with 4000 samples examined at NIFC. It allows the detection of the bacteria (Ag), whereas NIVR used ELISA to detect Ab. Apart from importing the diagnostic kit, VIFAVET used the kit that they produced on their own to diagnose the disease. The imported and own-produced kits were used by CP Jsc.

## Tests for Pasteurellosis

Table 20: Information on each test used for the detection of *Pasteurellosis*

Criterion	ELISA	PCR	Isolation	Test for antibiotic sensitivity
No. of tests/year/lab	50	10 - 200	3 -100	100
Sensitivity (agree/interviewees)	(1/1)*	High (1/3); (2/3)*	High (2/8); 70% (1/8); medium (1/8); (4/8)*	High (1/1)
Specificity (agree/interviewees)	(1/1)*	High (1/3); (2/3)*	High (2/8); medium (1/8) 40% (1/8); (4/8)*	Medium (1/1)
Cost	100	300	300 – 600	
Availability (agree/interviewees)		Yes (3/3)	Yes (6/8)	Yes
Feasibility (at central, local, farm level)	Central, local and farm (1/1)	Central (1/3); farm (1/3); (1/3)*	Central (1/8); local (4/8); farm (1/8); (2/8)*	Farm
Routine/request (%) (agree/interviewees)	0/100 (1/1)	0/100 (2/3); (1/3)*	0/100 (4/8); 20/80 (1/8) (3/8)*	20/80
Efficacy	*	High (1/3); (2/3)*	High (3/8); medium (2/8); low (1/8); (2/8)*	
Advantages	Detect Ab	Detect Ag (1/3)	Can obtain live bacteria; can detect the bacteria	
Disadvantages	No	(3/3)*	Need standard bacteria for culture	
Sources of test kit	Owned and imported (CP Jsc)	Imported	Imported (5/8); owned and imported (2/8) (CP and HUA); (1/8)*	Imported

\*=o not know

Four tests were used to detect Pasteurellosis; however, lower samples were examined. The maximum was 100 at HUA as well as at VIFAVET. Eight out of nine interviewees reported using the isolation test to detect the disease and the tested samples were mainly to accommodate requests. ELISA and -isolation test kits were own-produced and imported by CP and HUA.

## Tests for Salmonella spp.

*Salmonella* spp. was examined by seven laboratories of NIVR, HMU; PI-HCM, Sub-DAH-HCM, HUA, NIFC and NIN. The maximum number of samples (3000) was examined by isolation test at NIFC, which has the main function of detecting *Salmonella* spp. in pork in market places. Information on these tests are presented in Table 21.

Table 21: Tests used for detecting *Salmonella* spp.

Criterion	Isolation	Test for antibiotic sensitivity	PCR
No. of tests/year	100 – 3000	100	350
Sensitivity (agree/interviewees)	High (2/6); (4/6)*	High (1/1)	(1/1)*
Specificity (agree/interviewees)	High (1/6); Medium (1/6); (4/6)*	High	(1/1)*
Cost (x1000 VND)	458 (1/6); (5/6)*		*
Availability (agree/interviewees)	Yes	Yes	Yes
Feasibility	Central (1/6); local (1/6); (4/6)*	Central	Central
Routine/request (%) (agree/interviewees)	20/80 (1/6); 50/50 (1/6); (4/6)*	20/80	0/100
Efficacy	High (2/6); (4/6)*	High	*
Advantages	Can obtain live the bacteria	Effective treatment	*
Disadvantages	Need standard bacteria for culture	Time consuming	*
Sources of test kit	Own and imported (1/6); Imported (2/6); (3/6)*	Imported	Imported

\*=o not know

## Diagnostic tests for parasitic pig diseases

Table 22: Major parasitic pig diseases tested by institution/organization

Institution/organization	<i>Cysticercus cellulosae</i>	<i>Trichinella spiralis</i>	Fish-borne zoonotic trematodes	<i>Ascaris suum</i>	Toxoplasmosis
NIVR	√	√	√	√	√
HUA	√	√	√	√	
HMU		√		√	
NCVD		√		√	
CP Jsc.				√	

Parasitic diseases in pigs received attention from five institutions/organizations (4 were from government and only one from the private sector). Simultaneous with doing research, most of the major parasitic diseases in pigs were examined at NIVR and HUA while CP concentrated only on *Ascaris suum*. Fish-borne zoonotic trematodes (FZT) were included in this survey because pigs play an important role (especially in small-scale farms) in the transmission of FZT eggs to fishponds, which would put humans at risk if infected raw fish is consumed.

Eight diagnostic tests were used to detect pig parasitic diseases (Table 23).

Table 23: Tests used for detection of parasitic diseases

Test	<i>Cysticercus cellulosae</i>	<i>Trichinella spiralis</i>	Fish-borne zoonotic trematodes	<i>Ascaris suum</i>	Toxoplasmosis
ELISA	√	√			√
PCR	√	√	√	√	
Flotation method				√	
Sedimentation flotation and centrifugation			√		
Meat inspection	√				
Tissue digestion		√			
Morphological identification			√		
Histopathology	√	√			

Information on diagnostic tests used to detect parasitic pig diseases in each organization are given in Table 24.

Table 24: Diagnostic tests used to detect parasitic diseases in pigs

Organization	Test used							
	ELISA	PCR	Meat inspection	Histopathology	Tissue digestion	Flotation	Flotation, sedimentation and centrifugation	Morphological identification
NIVR	√	√	√		√	√	√	√
HUA			√	√				
HMU		√				√		
NCVD	√				√	√		
CP Jsc.						√		

Out of eight tests used to detect parasitic pig diseases, seven were used by NIVR. CP Jsc. used only the flotation method to detect *Ascaris suum*.

### Tests for *Cysticercus cellulosae*

For the detection of *Cysticercus cellulosae* in pigs, ELISA, PCR, histopathology, and meat inspection were used (Table 25). ELISA includes Ab-ELISA and Ag-ELISA.

Table 25: Tests used for detecting *Cysticercus cellulosae*

Criterion	ELISA	PCR	Histopathology	Meat inspection
No. of tests/year	1000	Depends on project	3000	100
Sensitivity (agree/interviewees)	High (2/2)	High (1/1)	(1/1)*	90% (2/2)
Specificity (agree/interviewees)	High (2/2)	High (1/1)	(1/1)*	90% (2/2)
Cost (x1000 VND)	50-100	500	100	
Availability (agree/interviewees)	Yes	Yes	Yes	Yes
Feasibility	Central and local (1/2)	Central	Central	Central (1/2); local (1/2)
Routine/request (%) (agree/interviewees)	0/100	0/100	0/100	0/100
Efficacy	High	High	Medium	High (1/2); medium (1/2)
Advantages	- Ag-ELISA (1/2): quick, accurate, detects circulating antigen of <i>Cysticercus cellulosae</i> -Ab-ELISA (1/2): high Se and Sp, detects Ab	High Se and Sp	Rather accurate	Cheap, simple
Disadvantages	-Ag-ELISA: expensive, cannot be applied in the field; need to check cross reaction with <i>C. tenuicollis</i> -Ab-ELISA: none	Cannot be applied in the field, require experience and equipment	Require experience and equipment	Experienced persons needed
Sources of test kit	-Ag-ELISA: imported -Ab-ELISA: Own-produced	Imported	Imported	Own (1/2); imported (1/2)

*C. cellulosae* was reportedly tested by NIVR, HUA, and NCVD (government entities). To detect *C. cellulosae* in pigs, ELISA that can detect either Ag or Ab was commonly used at NIVR. The number of samples/test/year mainly depends on the project. High Se and Sp were reported from ¾ of the tests. While PCR and histopathology methods originated from other countries, ELISA was own-produced. However according to an interviewee, these tests were only feasible in the laboratories and need to be modified for larger applications in the market or under field condition.

## Tests for *Trichinella spiralis*

Among the three institutions/organizations (NIVR, HUA, NCVD) that reported using diagnostic tests to detect *T. spiralis*, HUA was the only one organization that employed histopathology (Table 26).

Table 26: Tests used for the detection of *Trichinella spiralis*

Criterion	Ab-ELISA	Tissue digestion	Histopathology	PCR
No. of tests/year/lab	200-1000	Depends on project	3000	Depends on project
Sensitivity (agree/interviewees)	95% (1/3); 98% (1/3); High (1/3)	75% (1/2); Medium (1/2)	(1/1)*	(1/1)*
Specificity (agree/interviewees)	>90% (1/3); High (2/3); (1/3)*	High (2/2)	(1/1)*	*
Cost (x1000 VND)	50-120	100	100	*
Availability (agree/interviewees)	Yes	Yes	Yes	Yes
Feasibility	Central and local (1/3)	Central	Central	Central
Routine/request (%) (agree/interviewees)	0/100	0/100	0/100	0/100
Efficacy	High	Medium	Medium	High
Advantages	High Se and Sp, detects Ab, many samples can be examined; no cross-reaction with other parasites; positive and negative results are clearly separated; results are documented electronically	Less expense and less labor	Rather accurate	*
Disadvantages	Expensive, Ab cannot detected 3-5weeks after infection, cannot differentiate the infection caused by <i>Trichinella spiralis</i> from others <i>Trichinella spp</i> ; time-consuming; cannot be applied at field condition	Require experience and equipment	Require experience and equipment	*
Sources of test kit	Imported (2/3) Own-produced (1/3)	Imported	Imported	Imported

\*=o not know

Depending on project requirements, 200-1000 samples were tested for *T. spiralis* by ELISA, while 3000 samples/year, handled by undergraduate students at HUA, were tested by histopathology method. Even though high in Se and Sp, ELISA tests need to be modified so farmers or consumers can easily apply it (according to an interviewee).

## Tests for fish-borne zoonotic trematodes

Table 27: Tests used for detecting fish-borne zoonotic trematodes (FZT)

Criterion	Sedimentation + flotation and centrifugation	Morphological identification
No. of tests/year	200	200
Sensitivity (agree/interviewees)	High (1/1)	medium (1/1)
Specificity (agree/interviewees)	High (1/1)	Medium
Cost (x1000 VND)	50	50
Availability (agree/interviewees)	Yes	Yes
Feasibility	Central, local	Central
Routine/request (%) (agree/interviewees)	0/100	0/100
Efficacy	High	Medium
Advantages	Cheap, simple, quantitative, many samples can be handled, non-toxic chemical	Cheap, quantitative
Disadvantage	Need experience	Need experience and equipment
Sources of test kit	Own-produced and imported	Own-produced and imported

FZT was an emerging infectious disease and was a rather neglected disease in comparison with others. The abovementioned methods were applied at NIVR and description of the method was published in international journals (Anh, Phuong et al. 2008; Lan-Anh, Phuong et al. 2009; Nguyen, Nguyen et al. 2009)

## Vaccines used in the prevention of some pig diseases in Vietnam

Table 24: Vaccines used for pig diseases

Disease	Type of vaccine		No. sold/ year	Own- produced/ imported	Routine/ upon request	Challenges
	Live attenuated	Inactivated/ killed				
PRRS	√		13,000 – many	Imported	Routine	May cause wastage if some amount is left in the bottle
CSF	√	√	85,300 – many	Imported	Routine	May cause wastage if some amount is left in the bottle
FMD	√	√	13,125 – many	Imported	Routine	Side effect
Erysipelosis		√	13,140 – many	Both	Routine	Side effect
Mycoplasma Pasteurellosis		√	13,140	Imported Own- produced	Routine Routine	Side effect
Parvo virus		√		Imported	Routine	

Information on the vaccine used/produced were obtained from only three interviewees who come from DVS-TT, CP, and VIFAVET. The number presented in Table 24 was from DVS-TT who had received vaccines from DAH. Some reported a high number of vaccines sold per year while others did not know about that as well as the challenges involved.

In recent years, the number of pigs at any given time in Vietnam hovers between 26 and 27 million head, therefore, an estimation of total pigs in Vietnam can be near to a 100 million pigs/year. According to an expert who has worked for more than 20 years at the Division of Epidemiology of DAH, Vietnam at present does not have statistics related to how many pigs are due for vaccination. There are many reasons to explain the inadequacy of the data: the small scale of pig farms, lack of local veterinarians who can collect the information to report to the center, a complicated vaccine market, no system to collect and analyze data, and lack of reports from companies. Experts have identified four key pig diseases in Vietnam—these are CSF, PRRS, FMD, and Pasteurellosis. It was estimated that the number of vaccine doses needed for each disease would amount to 10 million a year. CSF vaccination was up to 80-90% due to government support programs.



# Limitations of some selected diagnostic tests in detecting pig diseases

The literature confirms the application of ELISA to detect many viral, bacterial or parasitic pig diseases. Ag-ELISA can detect directly the virus, bacteria, or parasite (e.g., Lan Anh et al 2014 used Ag-ELISA to detect *Fasciola* antigen from feces of cattle in Vietnam; Ag-ELISA was also used to detect and serotype FMDV (kit bought from the World Reference Laboratory in Pirbright)(Lan Anh, Thanh et al. 2014), while Ab-ELISA was used to detect an antibody against a specific antigen of a pathogen (Ab- ELISA kit from Prionic) was used to detect *Trichinella* spp. in Vietnam). However, it was known if Ab-ELISA could correctly reflect current infection status of the pathogen or could certainly indicate whether the antibody was from the disease or from vaccination. In some cases, the antibody could not be detected at the early stage after infection due to a lag in immunoresponse (Gamble 1996) (e.g., *Trichinella spiralis*). PCR allows directly detection of pathogens and it is a good test to confirm the presence of any pathogen.

Our inventory found that ELISA, PCR, and the isolation tests were mainly used in laboratories to diagnose pig diseases. However, some disadvantages were reported. Cross-reaction may occur between species of a pathogen genus when ELISA is applied (e.g., Ag-ELISA for detection of Cysticercosis in pigs or Ab-ELISA cannot differentiate vaccinated pigs from PRRS-infected pigs). It takes time to get results (a week) and many steps must be done before these are obtained. Other disadvantages include the high cost of imported kits; the requirement to have experienced lab technicians and have proper equipment available; and the risks related to the use of toxic chemicals. Some tests cannot be applied under field condition or in slaughterhouses and markets.

According to our consultant, real-time PCR has very high Se and Sp to detect the nucleic acid sequence of specific viral agents. However, the PCR test (both real-time and conventional) is rather expensive as the reagent is imported. Besides, equipment is expensive and needs annual calibration. Another matter is the need for PCR to validate be routinely in case the virus evolves, which can yield negative results because of PCR mismatch. So far, not all diseases can be diagnosed by RRT-PCR. For example, there is no RRT-PCR for serotyping FMDV.

For the diagnosis of bacterial diseases, the conventional (culture) method may be sufficient. However, there are still some constraints. First, this method takes time so there is no quick diagnosis. Besides, the injudicious use of antibiotics by farmers induce the culture method to fail to isolate the bacteria; they may find the bacteria resistant to the antibiotics used, but it is not the right causative agent. Some bacteria are not easy to grow on culturing media, e.g., *Mycoplasma hyopneumoniae*. Some bacteria may need specific enrichment media and selective media (*Salmonella* sp). After isolation, characteristic identification (biochemical and serological) is time-consuming and costly, and may not even be available. Some bacteria have inconsistent biochemical characteristics that make identification very difficult. Another method to identify the characteristics of bacteria is through PCR, but this assay is not available and established for many kinds of bacteria. In contrast to the diagnosis of viral diseases, in bacteriology, diagnosis using PCR cannot be done directly on the original specimen; an isolated colony must be used. So PCR is not really a rapid method to diagnose bacterial diseases.

## Potential diagnostic opportunities to be developed

With the quick development of pig husbandry practices and the proliferation of many kinds of pork products that are eaten raw, the risk of infection from pigs may have grave effects on human health. The habit of eating raw pork, fermented pork, and uncooked blood is common among the populace, especially during special occasions and days of celebration. Recent hospital reports from Hanoi showed hundreds of cases of *Cysticercosis* infection every year (Dorny et al., 2004). This may be an underestimation as most patients do not have access to hospitals. In addition, there were hundreds of cases of *Streptococcus suis* infection last year and there were reports of death from several areas in Vietnam.

Therefore, to control pig diseases, reduce the risks of transmission of zoonotic diseases, and improve public health, diagnostic laboratories need to be strengthened, and rapid test kits with high Se and Sp must be developed. These should be easily applied in the field and should have features that would enable farmers to recognize the disease in the farm so they can quickly respond or assist inspectors in slaughterhouses so they can right away examine the meat before they are sold in the market) or help consumers check the product before cooking or eating it. For example: developing a test strip to detect a parasitic disease from fermented pork (nem chua, nem tinh-source of *Cysticercosis*) or from raw pork (source of *Cysticercosis* and *Trichinellosis*) or from uncooked blood (tiet canh-source of *Streptococcus*) is very important. Coming up with a rapid kit to detect both bacterial and parasitic diseases is also critical (e.g., to detect *Salmonellosis* and *Cysticercosis* in fermented pork). In the future, training opportunities for relevant stakeholders in the value chain are essential because this is where knowledge and experiences are shared. Scientists from the BioScience Unit of ILRI may take the lead in furthering the techniques and scientific advances (e.g., purification of antigens, production of recombinant antigens, developing test strips) that are needed to detect and control the spread of pig diseases.

Recently, HUA has developed a rapid test to diagnose PRRS. These diagnostic kits are in pilot experiments. The same group desires to produce some more rapid test kits to detect circovirus (PCV2) in the future, however, budgets remain a constraint.

# Conclusions

Data from our inventory showed that

- Fourteen of 15 institutions/organizations were involved in the diagnosis of pig diseases. Most of the laboratories concentrate on detecting viral diseases, followed by bacterial and then parasitic diseases.
- To detect viral and parasitic diseases, ELISA and PCR tests are regularly used in laboratories of the government or private companies. The isolation test is regularly used to detect bacterial diseases. The number of tests conducted varies between institutions/organizations. Detailed information on Se and Sp of each test/pathogen is almost not available.
- The ELISA test can be used to detect the Ab or Ag of a pathogen. Many samples can be conducted at the same time, and the test is simple to perform. However, cross-reactions may occur and there is the problem of differentiating infected pigs from vaccinated ones. It is also time-consuming. In addition, experience and equipment are required so it has limited application in the field.
- PCR was reported to give accurate results and it can directly detect a pathogen. However, the test is expensive and it is only done upon request. Moreover, just like ELISA, it requires experience and equipment and could not be used under field conditions.
- The isolation test allows detection of bacterial/viral diseases but it is costly, time-consuming, and requires special media.
- Most of the test kits used are imported, except for some - e.g. VIFAVET produces their own kit to detect *E. coli septicemia*, while NIVR and NCVD produce kits to detect Ab against *C. cellulosa* or *T. spiralis*.
- Most of the interviewees emphasized the need for rapid and cheap diagnostic tests. When developed, test kits should be directly applied in the farms or used easily by farmers or consumers. Especially in demand are rapid diagnostic tests based on detecting DNA or RNA. Local veterinarians working in pig farms can be equipped with knowledge of rapid diagnostic tests that would give more accurate and faster results, thus avoiding wrong diagnosis. However, one interviewee thought that the existing tests are enough to diagnose pig diseases.
- Most of the interviewees were interested in answering the questions, except for a few who did not provide any information on the tests and vaccines used and did not share their insights on how to develop/improve these tests.
- There is high expectation to strengthen the capacity building efforts of ILRI with the end in view of developing improved and rapid diagnostic tests.

## Annex 1: Institutions/organizations engaged in detecting major pig diseases and the number of tests conducted per year

Institution/ organization	Major pig diseases							
	PRRS	CSF	FMD	E. coli septicemia	Pasteurella spp.	Salmonella spp.	C. cellulosae	T. spiralis
NIVR	500	100	300	1000	300	350	1000 or *	1000 or *
NCVD	1000	1000	70				100	200
HUA	1000	400	200	200	100	100	*	*
HMU			**	**		**		**
Sub-DAH- HCM	12850	5057	12924	**	**	**		
RAHO2	400	108	10		3			
RAHO6	8000	7000	6000					
NLU	**	**	**	**	**			
NIN				1000		310		
NIFC				4000		3000		
RTD	500	**	50					
CP Vietnam	18500	4000	1000	**	50			
VIFAVET	1100	1000		150	100			

\*Depends on the project; \*\*Does not remember

## Annex 2: Senior advisers consulted in this inventory

No	Name	Academic title	Organization/institution
1	Nguyen Viet Khong	Ass. Prof. PhD	National Institute of Veterinary Research
2	To Long Thanh	Ass. Prof. PhD	National Centre for Veterinary Diagnostics, DAH
3	Nguyen Tung	PhD	National Centre for Veterinary Diagnostics, DAH
4	Duong Van Nhiem	PhD	Faculty of Veterinary Medicine, HUA
5	Pham Duc Phuc	PhD	Hanoi School of Public Health

## Annex 3: Respondents interviewed

No	Name	Gender	Date of interview	Interviewer	Organization	Location	Position (lab experience in years)	Note
<b>Animal health</b>								
1	Nguyen Xuan Huyen Nguyen Thi Lan Anh	Male	2014.01.23	Vu Thi Kim Hue	National Institute of Veterinary Research	Hanoi	Vet (13)	Bacteria
2	Pham Thi Nga	Female	2013.12.24	Nguyen Tien Thanh	National Institute of Veterinary Research	Hanoi	Vet, Vice-head of Parasitology Dept (18)	Parasite
3	Nguyen Tung	Female	2013.12.27	Vu Thi Kim Hue	National Institute of Veterinary Research National Centre for Veterinary Diagnostics, DAH	Hanoi	Vet, Lab technician (7)	Virus
4	Vu Thi Nga	Male	2013.12.26	Nguyen Tien Thanh	National Centre for Veterinary Diagnostics, DAH	Hanoi	Vet, Vice director (18) Vice head of NCVD (10)	Laboratory diagnosis
5	Truong Van Minh	Female	2014.01.03	Nguyen Tien Thanh	DAH	Hanoi	(10)	Laboratory diagnosis
6	Vo Van Hung	Male	2013.12.30	Nguyen Tien Thanh	Regional animal health office 2, DAH	Hai Phong	Director (14)	Laboratory diagnosis
7	Vo Khac Tram	Male	2013.12.30	Vu Thi Kim Hue	Regional animal health office 6, DAH	Ho Chi Minh	Vet, Lab technician (5)	Laboratory diagnosis
8	Nguyen Duy Dang	Male	2014.01.23	Nguyen Tien Thanh	Sub - Department of Animal Health of HCM	Ho Chi Minh	Lab head (15)	Laboratory diagnosis
9	Nguyen Ngoc Hai	Male	2013.12.27	Nguyen Tien Thanh	Veterinary station of Thach That district, Hanoi	Hanoi	Head of DVS-TT	Farmer's perspective
10	Nguyen Huu Nam	Male	2013.12.30	Vu Thi Kim Hue	Nong Lam University, Ho Chi Minh City	Ho Chi Minh	PGS-TS (22)	University
11	Dao Huu Thong	Male	2013.12.27	Dang Xuan Sinh	Hanoi University of Agriculture	Hanoi	Senior teacher Consultant at farms (2)	University Lab and private company
12	Vu Thi Dung	Male	2013.12.23	Nguyen Tien Thanh	CP Group, Hanoi	Hanoi	Lab head (7)	Lab and private company
13	Huynh Bich Truyen	Female	2013.12.28	Nguyen Tien Thanh	CP Group, Hanoi	Hanoi	Lab head (7)	Lab and private company
14	Le Van Phan	Female	2013.12.28	Vu Thi Kim Hue	VIFAVET Company, Ho Chi Minh City	Ho Chi Minh	Vet, Lab technician (3)	Vet medicine private company
15		Male	2013.12.25	Vu Thi Kim Hue	RTD Company, HungYen	Hung Yen	Vet (14)	Vet medicine private company
<b>Public health</b>								
16	Nguyen Thi Giang	Female	2013.12.27	Luu Quoc Toan	National Institute of Food Control	Hanoi	Doctor (8)	Public health, zoonosis
17	Bui Thi Mai Huong	Female	2013.12.27	Luu Quoc Toan	National Institute of Nutrition	Hanoi	Doctor (17)	Public health, zoonosis
18	Nguyen Vu Trung	Male	2014.01.10	Luu Quoc Toan	Hanoi Medical University	Hanoi	Doctor (18)	Public health, zoonosis
19	Cao Thi Bao Van	Female	2014.03.27	Nguyen Tien Thanh	Pasteur Institute, Ho Chi Minh City	Ho Chi Minh	Doctor, Vice director (27)	Public health, zoonosis

# Annex 4: Questionnaire and guidelines

Discussion guide for key informants of available diagnostic techniques of pig diseases in Vietnam, 2013

This interview relates to a planned inventory of available diagnostic tools for selected key pathogens along the pig value chain in Vietnam. It will identify gaps and needs to develop or adapt currently available tools to the local context. It is also anticipated that this will contribute to future proposals for strengthening animal health services. The interview will take approximately 50 minutes. This interview will be recorded, unless you object.

This process ensures is anonymity. Your name and other identifying information will not be used in summary reports. The results of this questionnaire will be published in a report and presented to various stakeholders. Although identifying information was needed from those who choose to participate in these interviews, information will be kept confidential and will not be included in any public report.

**Objective:** Understanding and listing all the available diagnostic tools of diseases along the pig value chain in Vietnam. Analyzing these tools to identify gaps and needs to develop or adapt currently available tools to the local context. Respondents are technicians, lab managers, and experts who have a good understanding of pig production and pig health.

## I. General information

1. First of all, I would like you to introduce yourself (Name, age, professional, expertising, job title, experience on lab work, years, name and type of organization,...)

2. Which of these areas is your organization/lab/company engaged in?

Animal diseases/production

Public health

Food safety

Others, ask for details \_\_\_\_\_

3. Which reference status has your lab obtained?

International standard

National standard

Local standard

If yes, ask for details: \_\_\_??? but no question here that is answerable by yes or no \_\_\_\_\_

4. Is your organization involved in diagnostics only or also in prevention, including vaccination?

Diagnostic function

Prevention function

If yes, provide details: .....

(Others), provide details: .....

5. Is your lab involved in regulation development or implementation?

Yes

No

How is the lab involved?

---

6. Does your lab contribute to policy development or implementation?

Yes

No

How is the lab involved?

---

7. What kind of pathogens do you/ your lab focus on?

Virus

Bacteria

Parasites

Other hazards: .....



8. Can you list all pig diseases (both done routinely and upon request) that you/your lab is working with? How often did you see positive tests/tests applied to the mentioned pathogens during the last year?

Disease	Routine	On request	Never	No. of tests/ year	No. of positive tests
PRRS*					
Classic swine fever*					
Food and mouth disease*					
E. coli septicemia*					
Erysipelosis*					
Pasteurellosis*					
Paratyphoid suum disease*					
Mycoplasmosis					
Other					
Salmonellosis					
Campylobacteriosis					
Streptococcus suis disease*					
Leptospirosis*					
Japanese encephalitis					
Brucellosis					
(H1N1)*					
Other					
Parasitic/others					
Endo-parasitosis					
Roundworms					
Ecto-parasitosis					
Mange					
Other					
Cysticercus cellulosae*					
Trichinella spiralis *					

II. Information on vaccination applied or produced related to selected pathogens

Disease	Type of vaccine	No. sold /year	Own-produced	Imported	Routine/request	Known challenges (e.g. cross reaction, protection, costly, cool? chain,...)
PRRS*						
Classic swine fever*						
Food and mouth disease*						
E. coli septicemia*						
Erysipelosis*						
Pasteurellosis*						
Paratyphoid suum disease*						
Mycoplasmosis						
Other						
Salmonellosis						
Campylobacteriosis						
Streptococcus suis disease*						
Leptospirosis*						
Japanese encephalitis						
Bruccellosis						
(H1N1)*						
Other						
Parasitic/others						
Endo-parasitosis						
Roundworms						
Ecto-parasitosis						
Mange						
Other						
Cysticercus cellulosae*						
Trichinella spiralis *						

Note: Demonstrate the challenges of vaccine use as much as possible

III. Specific test used for certain pathogens

Note: Respondents may provide more than one test per pathogen. You may limit responses here to the three most frequently used tests.

Disease	Test*	No of tests/year	Sensitivity	Specificity	Cost (VND)/ test Who pays?	Availability	Feasibility (central/local/farm)	Routine/ request (%)	OIE recommended	Specimen used	Efficacy (high/ medium/ low)	Advantages**	Constraints of test	Origin of test***
PRRS*														
Classic swine fever*														
Food and mouth disease*														
E. coli septicemia*														
Erysipelosis*														
Pasteurellosis*														
Paratyphoid suum disease*														

Mycoplasmosis														
Other														
Salmonellosis														
Campylobacteriosis														
Streptococcus suis disease*														
Leptospirosis*														
Japanese encephalitis														
Brucellosis														
(H1N1)*														
Other														

Parasitic/others														
Endo-parasitosis														
Roundworms														
Ecto-parasitosis														
Mange														
Other														
Cysticercus cellulosae*														
Trichinella spiralis *														

\*Available diagnostic tools (rapid test, paper test, ELISA, PCR, Realtime PCR, culture,...)

\*\*Advantages of test (what is good with the tests?)

\*\*\*Origin of test (Materials to be used to do the test are produced by the lab, imported, or by company in Vietnam)

- IV. Probing the advantages and disadvantages of each technique in the tables above (*should refer also to a pathogen and to the table above*)
9. Are there any cross contamination/reaction due to vaccination or concurrent infections? (*false positive, negative*)
10. How could each diagnostic test mentioned in the table above be improved or developed? Why? How?
11. If you are to develop new diagnostic tools, what types of tools do you want to develop, adapt or improve from the existing tools (more rapid, cheaper diagnostic tools [e.g. filter paper test kits, etc.]?)
12. In your opinion, who would be the most interested in using the test results? (e.g., lab technicians, farmers, or field vets) and why (*e.g., cheap, no cool chain...*) ?

## Annex 5: References

- Anh, N. T., N. T. Phuong, G. H. Ha, L. T. Thu, M. V. Johansen, D. K. Murrell and S. M. Thamsborg (2008). "Evaluation of techniques for detection of small trematode eggs in faeces of domestic animals." Vet Parasitol **156**(3-4): 346-349.
- Chien, N. V. (2013). Báo cáo kết quả thực hiện kế hoạch 5 tháng năm 2013 ngành nông nghiệp và phát triển nông thôn: 16.
- Gamble, H. R. (1996). "Detection of trichinellosis in pigs by artificial digestion and enzyme immunoassay." J Food Prot **59**(3): 295-298.
- Lan-Anh, N. T., N. T. Phuong, K. D. Murrell, M. V. Johansen, A. Dalsgaard, L. T. Thu, T. T. Kim-Chi and S. M. Thamsborg. (2009). "Animal reservoir hosts and fish-borne zoonotic trematode infections on fish farms, Vietnam." Emerg Infect Dis **15**(4): 540-546.
- Lan Anh, N. T., D. T. Thanh, D. H. Hoan, T. Thuy do, N. V. Khong and N. Anderson (2014). "The transmission of *Fasciola* spp. to cattle and contamination of grazing areas with *Fasciola* eggs in the Red River Delta region of Vietnam." Trop Anim Health Prod **46**(4): 691-696.
- Nguyen, T. L., T. P. Nguyen, M. V. Johansen, K. D. Murrell, T. V. Phan, A. Dalsgaard, T. T. Luong and S. M. Thamsborg. (2009). "Prevalence and risks for fishborne zoonotic trematode infections in domestic animals in a highly endemic area of North Vietnam." Acta Trop **112**(2): 198-203.