Rapid Diagnosis of African Swine Fever

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China Animal Disease Control Center

Outline

- A review of diagnostic techniques
- Pathogen detection technologies
  - *Molecular biology technology*
  - *Immunological technology*
  - *recommended detection techniques*
- Antibody detection technologies

1. ASF DIAGNOSTIC TECHNIQUES:

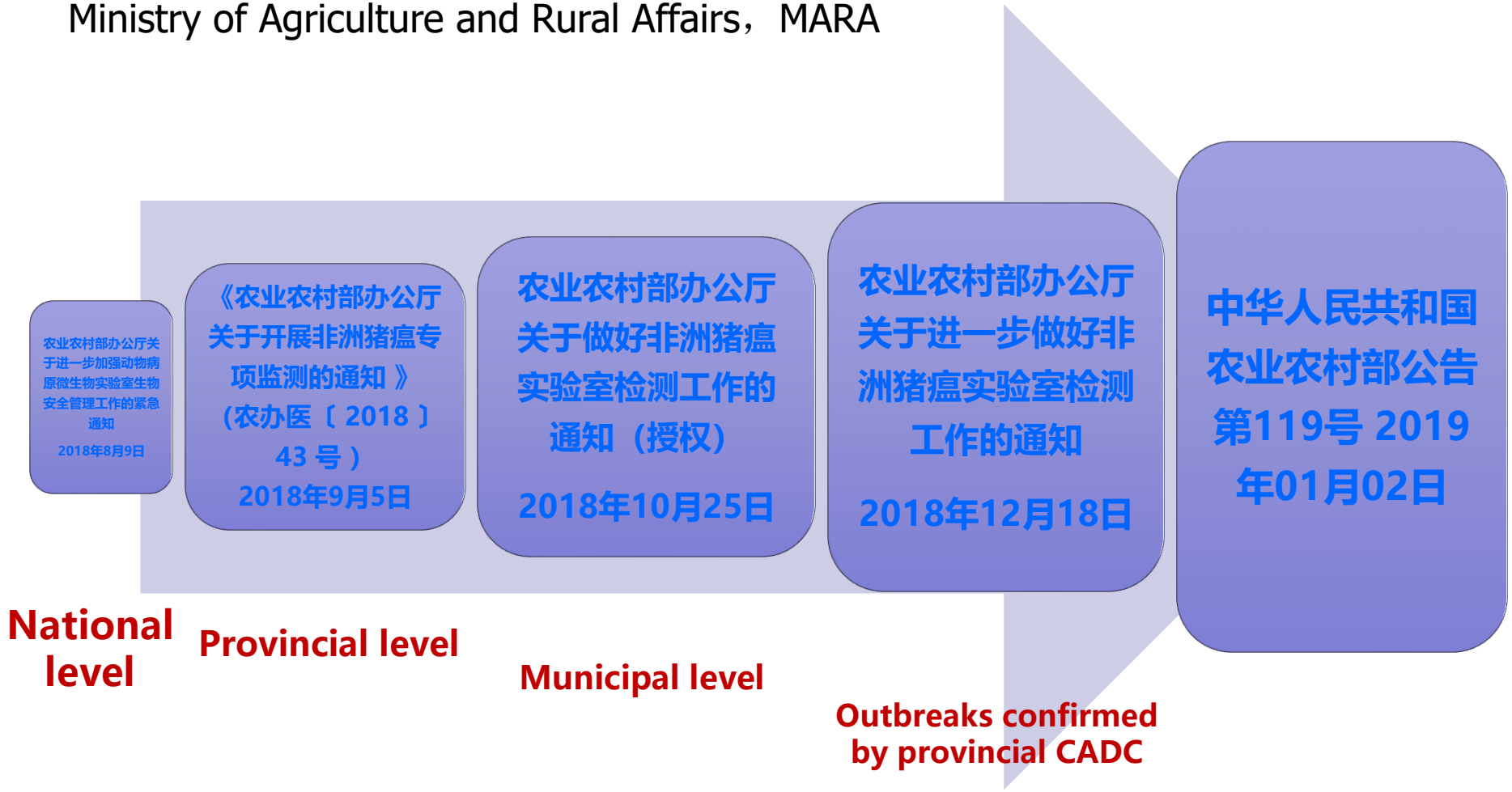
A brief review

1.1 ASF diagnostic techniques

- ASFV
  - Virological
    - Virus isolation
    - Direct IF
    - PCR
      Real-time PCR
    - Sandwich ELISA
    - Test strip
  - Antibody
    - Indirect ELISA
    - Blocking ELISA
    - IB
    - Immunofluorescence
    - Immunoperoxidase
    - Test strip

1.2 The Change Process of Detection Policy

Ministry of Agriculture and Rural Affairs，MARA



2. VIRUS DETECTION ASSAYS:

A brief introduction

2.1 DETECTION OF ASF VIRUS

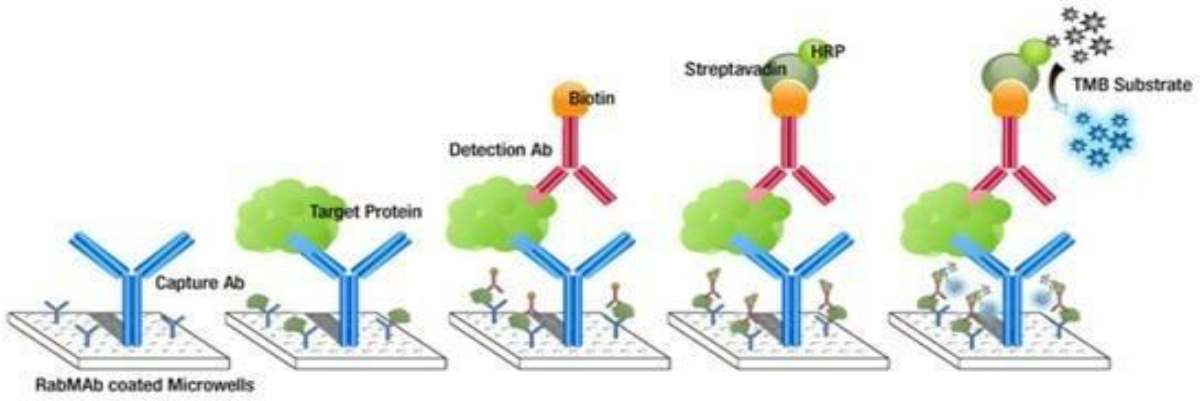
Positive result indicates ongoing infection

ASFV identification	VI-HAD	tissue exudate（spleen, lymph nodes, liver, tonsil, heart, lung, and kidney), blood, soft tick samples
	DIF	tissue（same to VI-HAD), blood, soft tick samples, cell cultures of ASFV
	ELISA	Same to VI-HAD
	IEH	Same to DIF
	Genome detection	tissue exudate（same to VI-HAD, putrefaction tissue, Bone marrow), blood, filter paper, soft tick, cell cultures of ASFV

Real-time PCR is the most widely used method for ASFV detection, allowing a sensitive, specific, and rapid result.

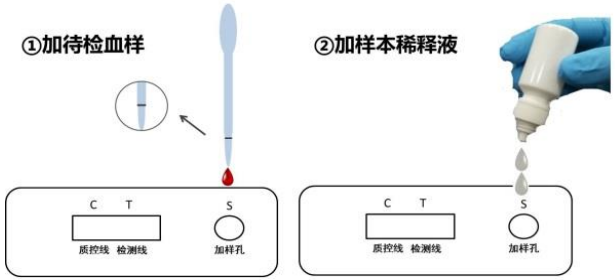
2.2 Virological detection tests -ELISA

- Allows large-scale testing of samples in a short time without special laboratory equipment.
- For subacute and chronic, sensitivity decreased significantly(50-500HAD_{50}/mL)
- Field samples in poor condition also decrease the sensitivity.
- It is thus recommended to only as a “herd” test .



2.3 Virological detection tests - Lateral Flow Assay (LFA)

- Developed by SASTRE using MAbs against VP72 protein of ASFV in 2016.
- Sensitivity similar to that of a commercially available antigen-ELISA, while the PCR always showed to be more sensitive
- With the field samples, the PCR detected more positives than LFA.



We have developed LFA using Mabs against VP72.

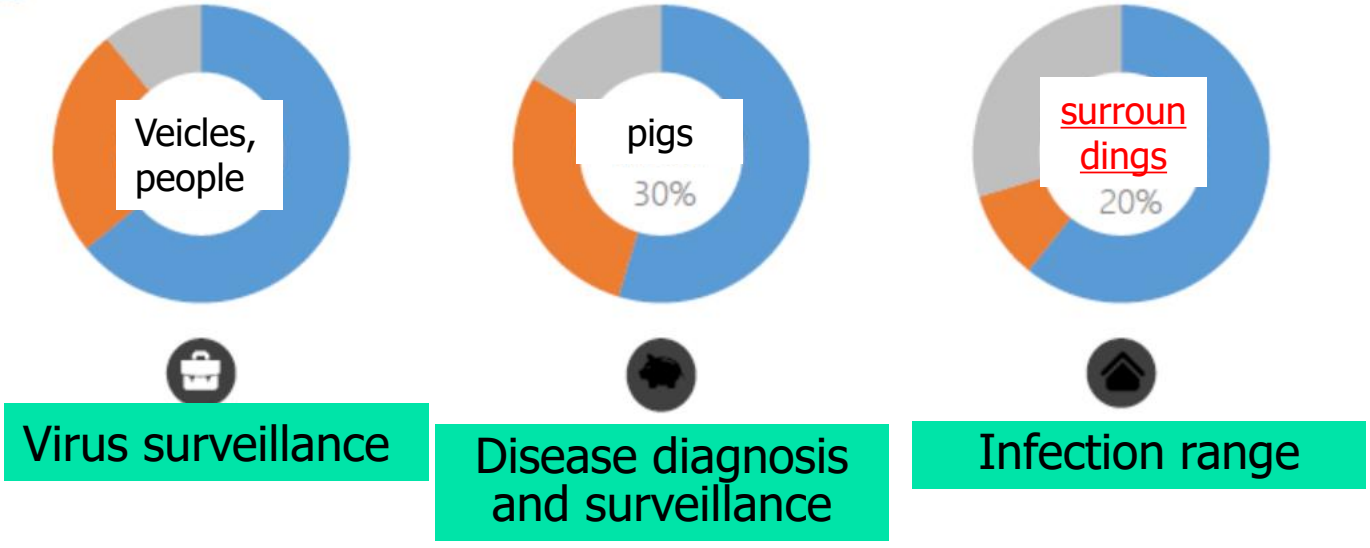
2.4 Nucleic acid detection

- **PCR:** Complex operation， easy to get contamination
- **Real-time PCR:** expensive equipment, sensitivity, high-throughput, and specificity
- **Isothermal amplification:** common equipment, high cost, low sensitivity
- **Microfluidics:** costly

PCR and Real-time PCR were recommended by OIE and FAO as optimal methods for ASFV detection

Types of sample

- Body fluids: saliva, tears, nasal secretions, blood, semen
- Tissues: spleen, lnymph nodes, lungs, liver, kidney, meat, tonsil.
- Excreta: feces, urine
- Samples should be inactivated after detection

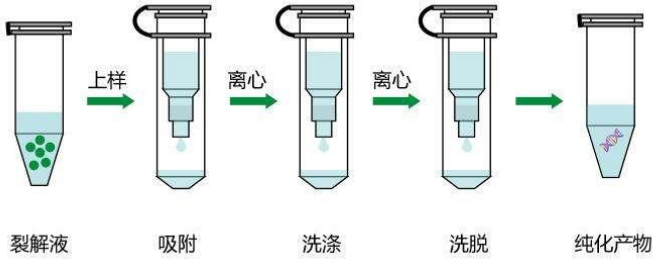


What type of samples to collect and how to collect?? Considering virus load, convenience, etc.

2.4.1 Real-time PCR

- Higher sensitivity and specificity than conventional PCR
- Closed reacting-Avoid cross-contamination
- More than 20 samples mix

1. DNA extraction

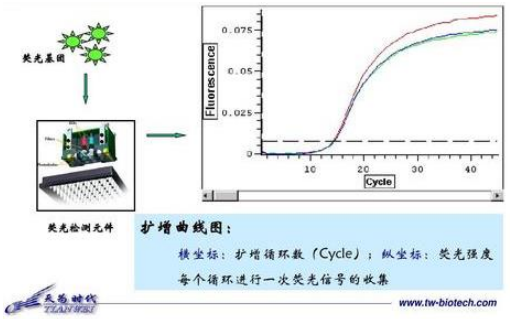


25 min

2. Adding samples and Amplification

60 min

3. Results analysis



5 min

2.4.2 Rapidly Real-time PCR (with or without extraction)

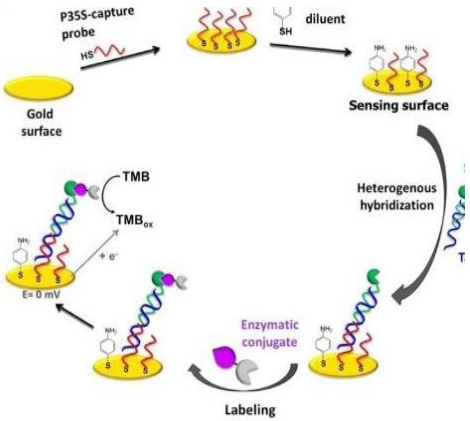
- **Detection time should not exceed 1h**
- **Easily carried out on slaughterhouse**

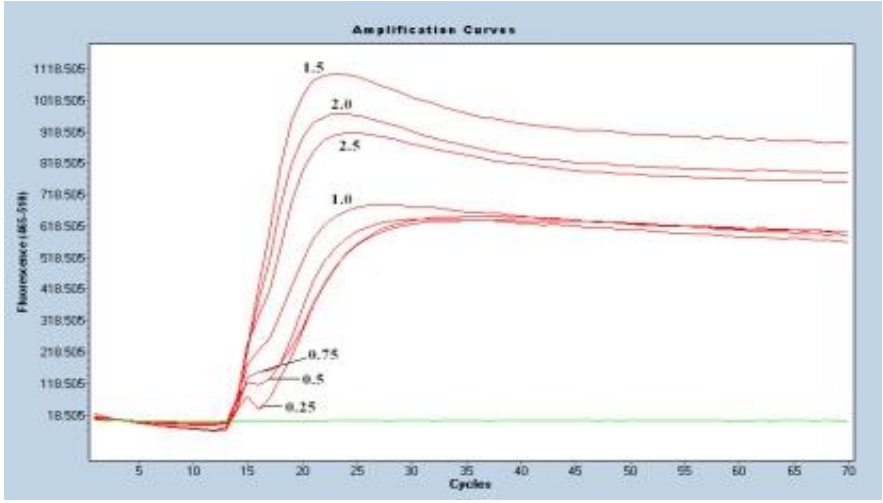
1. DNA automatic extraction or extraction-free

2. Quickly amplification and analysis

2.4.3 Real-time isothermal amplification detection kit

- **Without extraction， shortened time to 15 minutes**
- **Sensitivity and specifity, 95% consistency with rPCR.**
- **Amplified with consistent temperature at 64℃**

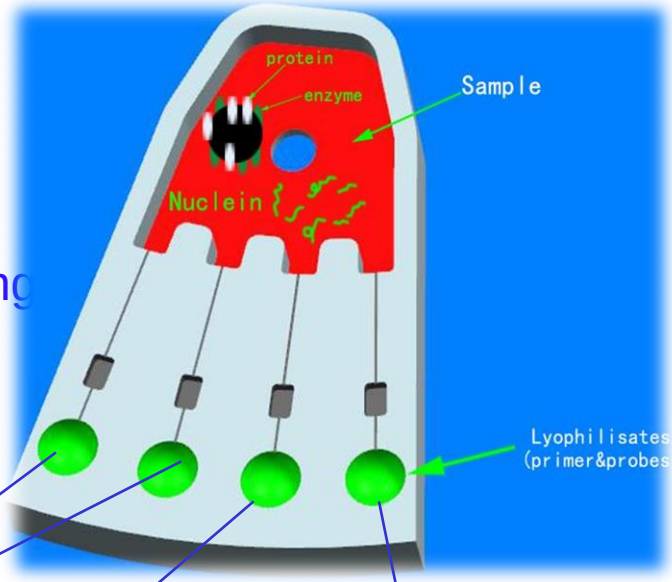




Samples suitable for rapid extraction or extraction-free

Sample Types	Sample	DNA preparison
Blood	Blood, serum, semen, saliva, urine, etc.	With or without extraction
Immune organs	Lymphoid node, spleen	With or without extraction
Meat, meat products	meat	Rapid Extraction or extraction
Fat meat	Head, trotters, fat, lard etc.	Extraction
Surroundings	Dust, feed, sewage, feces, etc.	Extraction

2.4.4 Microfluidics



Target Naïve control Internal standard positive control

- Fast detection, results in 30 minutes
- Primer pre-buried, sample loading steps simplified
- Multi-hole detection, no need for re-examination, higher accuracy for low concentration
- Internal standard quality control, no external independent control
- Detection cost reduced by 13-25%

2.5 Nucleic acid detection methods

Methods	Nucleic acid extraction	Amplification	Observation	Total time	Specificity	Sensitivity
Conventional PCR	≈25min	≈ 60min	≈ 30min	≈ 115min	★★★	★★
Real-time PCR	≈ 25min	≈ 60min	/	≈ 85min	★★★	★★★
Rapidly Real-time PCR（without extration）	≈5min	≈ 30min	/	≈35min	★★★	★★
Rapidly Real-time PCR（with extration）	≈ 25min	≈ 30min	/	≈55min	★★★	★★★
RPA/RAA	≈ 25min	≈15min	/	≈40min	★★	★★★
LAMP	≈ 25min	≈20min	/	≈45min	★★	★★★
Microfluidics	≈ 25min	≈ 35min	/	≈ 55min	★★	★★

2.6.1 Evaluation and recommendation of ASFV detection kits, organized by MARA in China (contd.)

In November 2018 and June 2019, the MARA organized evaluation of two batches of ASF rapid detection kits for recommendation to use.

A total of 43 (1st batch) and 93 (2nd batch) kits were

Evaluation

Including: real-time PCR, LAMP kits, Test strips, and micro fluid







2.6.2 Evaluation and recommendation of ASF detection kits, organized by MARA, China

- **Definition (in China) of rapid diagnostic techniques**
  - Detection time should not exceed 1h
  - **Easily carried out without special training**
  - Reagent type
    - Nucleic acid detection kit
    - ELSA Kit for Antigen Detection
    - Antigen rapid test strip

- **Application scope of rapid diagnostic techniques**
  - Detection of ASFV in slaughterhouses
  - Rapid detection of African swine fever in pig herds
  - On-site rapid detection

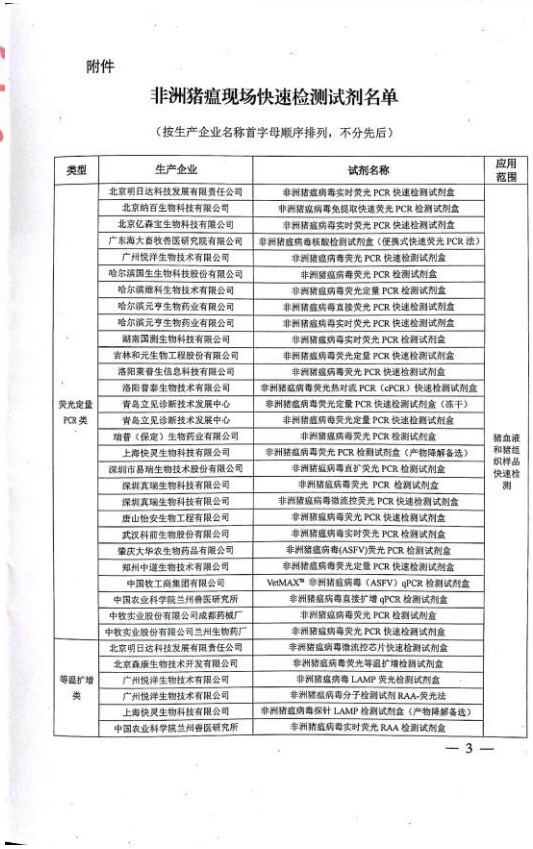
2.6.3 Evaluation and recommendation of virological detection tests for ASFV organized by MARA in China

Eventually, a total of 11 and 34 kits were recommended in nationwide, respectively, including real-time PCR kits, isothermal amplification kit, and micro. These kits are currently used widely in China.

- Detection limit
- Specificity
- Sensitivity
- Repeatability
- Detection time
- Simplicity
- Practicability

中国动物疫病预防控制中心
（农业农村部屠宰技术中心

疫控（诊）〔2019〕3 号



附件

非洲猪瘟现场快速检测试剂名单

（按生产企业名称首字母顺序排列，不分先后）

类型	生产企业	试剂名称	应用范围
荧光定量PCR类	北京明日达科技发展有限责任公司	非洲猪瘟病毒实时荧光 PCR 快速检测试剂盒	猪血液和猪组织样品快速检测
	北京纳百生物科技有限公司	非洲猪瘟病毒免提取快速荧光 PCR 检测试剂盒	
	北京亿森宝生物科技有限公司	非洲猪瘟病毒实时荧光 PCR 快速检测试剂盒	
	广东海大畜牧兽医研究院有限公司	非洲猪瘟病毒核酸检测试剂盒（便携式快速荧光 PCR 法）	
	广州悦洋生物技术有限公司	非洲猪瘟病毒荧光 PCR 快速检测试剂盒	
	哈尔滨国生生物科技股份有限公司	非洲猪瘟病毒荧光 PCR 检测试剂盒	
	哈尔滨维科生物技术有限公司	非洲猪瘟病毒荧光定量 PCR 检测试剂盒	
	哈尔滨元亨生物药业有限公司	非洲猪瘟病毒直接荧光 PCR 快速检测试剂盒	
	哈尔滨元亨生物药业有限公司	非洲猪瘟病毒实时荧光 PCR 快速检测试剂盒	
	融南国测生物科技有限公司	非洲猪瘟病毒实时荧光 PCR 检测试剂盒	
	吉林和元生物工程股份有限公司	非洲猪瘟病毒荧光定量 PCR 快速检测试剂盒	
	洛阳莱普生信息科技有限公司	非洲猪瘟病毒荧光 PCR 快速检测试剂盒	
	洛阳普泰生物技术有限公司	非洲猪瘟病毒荧光热对流 PCR（cPCR）快速检测试剂盒	
	青岛立见诊断技术发展中心	非洲猪瘟病毒荧光定量 PCR 快速检测试剂盒（冻干）	
	青岛立见诊断技术发展中心	非洲猪瘟病毒荧光定量 PCR 快速检测试剂盒	
	瑞普（保定）生物药业有限公司	非洲猪瘟病毒荧光 PCR 检测试剂盒	
	上海快灵生物科技有限公司	非洲猪瘟病毒荧光 PCR 检测试剂盒（产物降解备选）	
	深圳市易瑞生物技术股份有限公司	非洲猪瘟病毒直扩荧光 PCR 检测试剂盒	
	深圳真瑞生物科技有限公司	非洲猪瘟病毒荧光 PCR 检测试剂盒	
	深圳真瑞生物科技有限公司	非洲猪瘟病毒微流控荧光 PCR 快速检测试剂盒	
	唐山怡安生物工程有限公司	非洲猪瘟病毒荧光 PCR 快速检测试剂盒	
	武汉科前生物股份有限公司	非洲猪瘟病毒实时荧光 PCR 检测试剂盒	
	肇庆大华农生物药品有限公司	非洲猪瘟病毒(ASFV)荧光 PCR 检测试剂盒	
	郑州中谨生物技术有限公司	非洲猪瘟病毒荧光定量 PCR 快速检测试剂盒	
	中国牧工商集团有限公司	VetMAX™ 非洲猪瘟病毒（ASFV）qPCR 检测试剂盒	
	中国农业科学院兰州兽医研究所	非洲猪瘟病毒直接扩增 qPCR 检测试剂盒	
	中牧实业股份有限公司成都药械厂	非洲猪瘟病毒荧光 PCR 检测试剂盒	
	中牧实业股份有限公司兰州生物药厂	非洲猪瘟病毒荧光 PCR 快速检测试剂盒	
等温扩增类	北京明日达科技发展有限责任公司	非洲猪瘟病毒微流控芯片快速检测试剂盒	
	北京森康生物技术开发有限公司	非洲猪瘟病毒荧光等温扩增检测试剂盒	
	广州悦洋生物技术有限公司	非洲猪瘟病毒 LAMP 荧光检测试剂盒	
	广州悦洋生物技术有限公司	非洲猪瘟病毒分子检测试剂 RAA-荧光法	
	上海快灵生物科技有限公司	非洲猪瘟病毒探针 LAMP 检测试剂盒（产物降解备选）	
	中国农业科学院兰州兽医研究所	非洲猪瘟病毒实时荧光 RAA 检测试剂盒	

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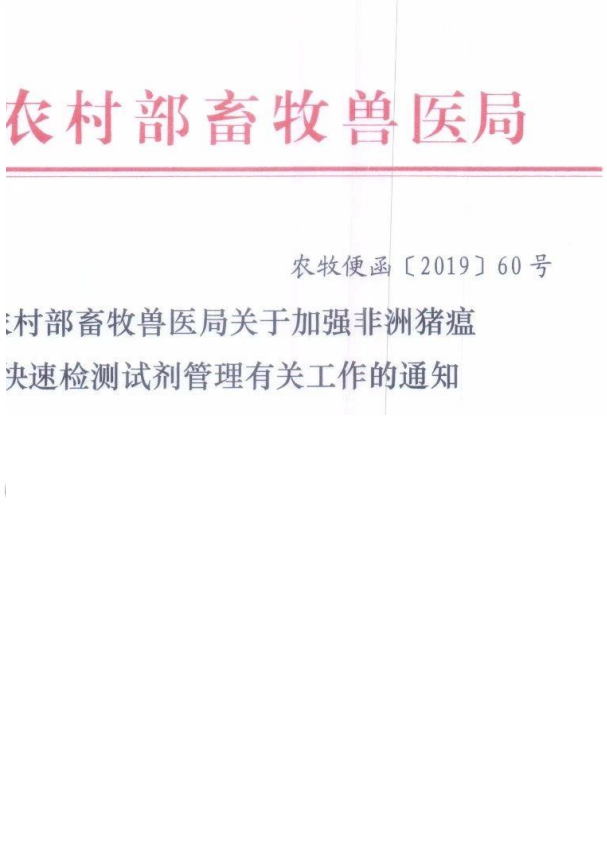


农村部畜牧兽医局

农牧便函〔2019〕60 号

村部畜牧兽医局关于加强非洲猪瘟
快速检测试剂管理有关工作的通知

The first licensed real-time PCR kit for ASFV in China

中华人民共和国

新兽药注册证书

证号：（2019）新兽药证字 11 号

新兽药名称：非洲猪瘟病毒荧光PCR检测试剂盒

注册分类：二 类

研制单位：中国动物疫病预防控制中心、北京森康生物技术开发有限公司、洛阳莱普生信息科技有限公司、深圳真瑞生物科技有限公司

根据《兽药管理条例》，该兽药符合规定，准予注册，特发此证。

发证日期：二〇[illegible]年[illegible]月[illegible]三日

中华人民共和国农业农村部

兽药产品批准文号批件

批件号：07040020190308-065

企业名称：北京森康生物技术开发有限公司

生产地址：北京市怀柔区杨宋镇凤翔一园23号

通用名称：非洲猪瘟病毒荧光PCR检测试剂盒

商品名称：无

根据《兽药管理条例》《兽药产品批准文号管理办法》等有关规定，该产品经审查符合要求，准予生产。

兽药产品批准文号：兽药生字010628858

含量规格：48检测/盒

有效期：2019-04-17至2024-04-16

发证日期：2019-04-17

Licensed last year by the MARA in China

We didn't recommend antigen-ELISA and LFA for ASFV detection in China, why??

- 1. Lower sensitivity than nucleic acid detection, i.g. false negative up to 48% for antigen-ELISA and 60% for LFA;
- 2. Instable specificity for antigen-ELISA, and hard to achieve high sensitivity and specificity simultaneously;
- 3. Time cost: antigen-ELISA costs 3-fold longer time than real-time PCR.

Here are reasons:

3. DETECTION OF ASF ANTIBODIES

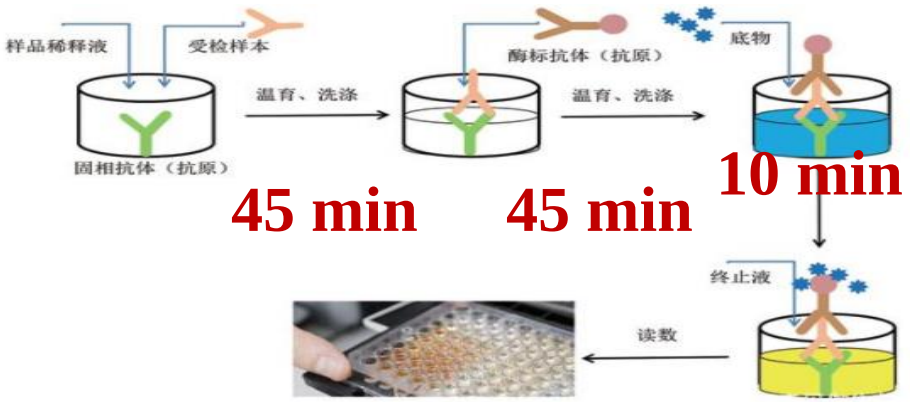
3.1 Summary of antibodies detection assays

Current or historic infection

Methods	ELISA	IIF	IB	IEOP
Applied range	Subacute or chronic infections, large-scale screening test	Confirmatory test	Confirmatory test	large-scale serological studies
Comment	Not suitable for acute or acute infections, need to be confirmed	Not suitable for Screening	Not suitable for Screening	Need to be confirmed
Time	3h	2h	3h	30min

3.2 Enzyme-Linked Immuno Sorbent Assay (ELISA)

- Simple operation and low equipment requirements
- High speed
- Low costs
- Easy interpretation
- No cross-contamination
- Suitable for elimination or eradication project
- Large scale screened thanks to the automatic equipment available.



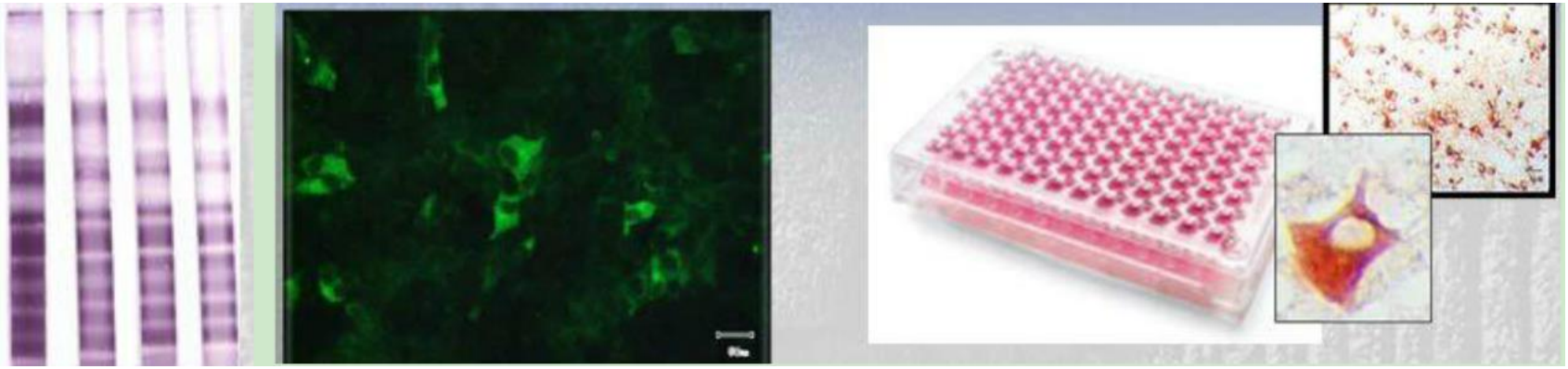
Detection time about 2.5h

Techniques not listed by OIE as confirmatory ones

- A variety of commercial and “in-house” methods such as indirect or competitive ELISA tests are currently available for ASF antibody detection.
- Sera incorrectly handled or badly preserved (due to inadequate storage or transportation) and haemolyzed samples may yield up to 20% false-positive results.
- All positive and doubtful samples by ELISA must be confirmed by alternative serological confirmatory tests.

3.3 Other Antibody Diagnosis Methods

- **Immunoblotting（IB）: semi-purified viral antigen**
- **Indirect immunofluorescence (IFI): passage cells infected with ASFV**
- **Immunoperoxidase assay (IPT): virus-infected cells**
- **Not routinely used**



Thank you !