

How to Investigate a Suspect Case of Infectious Disease

Diagnosis of Equine Infectious Diseases

Prof. Wang Xiaojun

Harbin Veterinary Research Institute, CAAS, China

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SPF Animals Facilities
(8,000 m²)

R&D Department
(8,000 m²)

Vaccine Production
Workshops
(35,000 m²)

Scientific Complex
(46,000 m²)



Assistant Facilities

Animal Facilities
(13,000 m²)

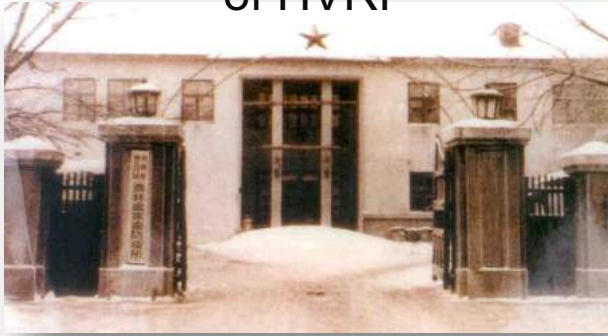
Biology Safety Laboratory
(20,000 m²)

Visiting Center
(18,000 m²)

Veterinary Graduate School (20,000 m²)

Milestones of HVRI

Original location
of HVRI



Founded on 1st, June.
The first veterinary
research institute in China



1948

1957



Subordinated to Chinese Academy of
Agriculture since 1957



HVRI Old Building

State Key Laboratory of
Veterinary Biotechnology



State Key Laboratory of Veterinary
Biotechnology , the first state key lab of
veterinary in China



1986

1992



Harbin Weike Biotechnology
Development Company , 1992



Harbin Weike Biotechnology
Development Company

National Engineering Research
Center for Veterinary Biologics



National Engineering Research Center for Veterinary
Biologics, the first state-owned engineering research
center for veterinary products



2010

2015



The new campus of HVRI
Veterinary Graduate School



HVRI New Building



- State Key Laboratory of Veterinary Biotechnology
- National High-level Biosafety Laboratory for Animal Disease Prevention and Control
- National Avian Influenza Reference Laboratory
- National Bovine Infectious Pleuropneumonia Reference Laboratory
- National Glanders Reference Laboratory
- National Reference Laboratory for Equine Infectious Anemia
- FAO Animal Influenza Reference Center
- OIE Avian Influenza Reference Laboratory
- OIE Reference Laboratory for Equine Infectious Anemia
- OIE Infectious Bursal Disease Reference Laboratory
- OIE Asia-Pacific Zoonoses Collaboration Center

Research Directions of the State Key Laboratory of Veterinary Biotechnology



Comparison with SCI articles of similar high-level research institutions abroad (2011-2015)

	Status and influence	No. of articles	Frequency of citations by others	H-index
State Key Laboratory of Veterinary Biotechnology (SKLVB)	Excellent State Key Laboratory	397	1630	14
Cornell University School of Veterinary Medicine (CVM, Cornell University)	No. 1 in the U.S. Veterinary Specialty	134	433	10
USDA National Animal Disease Center (NADC , USDA)	U.S. disease prevention and control authority	14	86	6
Pirbright Institute, UK	The world's leading veterinary institute	385	2683	23
Australian Animal Health Laboratory (AAHL)	The world's top biosafety research institute	212	1280	14

Innovative Engineering Training Teams-6 Excellent Teams

Lab innovation team

Animal flu



Chen Hualan

Poultry immunosuppressive disease



Wang Xiaomei

Porcine Infectious Disease



Qiu Huaji

Porcine Digestive Tract Infectious



Feng Li

Zoonoses and exotic diseases



Bu Zhigao

Equine disease



Wang Xiaojun

Avian respiratory infections



Liu Shengwan

Zoonotic diseases of natural foci



Qu Liandong

Cattle disease



Yu Li

Animal bacterial disease



Liu Si

Animal mycoplasmosis



Xin Jiani

Basic immunity



Weng Chao

Epidemiology and Informatics



Cai Yan

Laboratory Animals and Comparative Medicine



Chen H

Pathogen structure and biological information



Wang Jiefu



Harbin Veterinary Research Institute, CAAS

Equine infectious disease and lentiviral disease research team

- ◆ State Key Laboratory of Veterinary Biotechnology
- National Glanders Reference Laboratory
- National Reference Laboratory for Equine Infectious Anemia
- ◆ OIE Reference Laboratory for Equine Infectious Anemia

国家马传染性贫血
参考实验室

National Equine Infectious Anemia Reference Laboratory

国家马鼻疽
参考实验室

National Glanders Reference Laboratory



Member of Equine Infectious Disease and Lentiviral Disease Research Innovation Team

Basic Research

Applied Research



Wang Xiaojun
Researcher

Chief Scientist



Lin Yuezhi
Associate
researcher

EIAV



Guo Wei
Associate
Researcher

Flu



Hu Zhe
Associate
Researcher
vaccine
diagnosis



Du Cheng
Associate
Researcher

EIAV



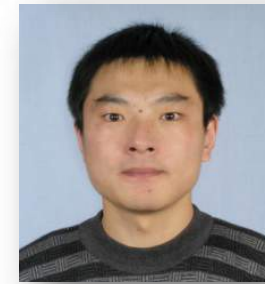
Wang
Xuefeng
Associate
Researcher

EIAV

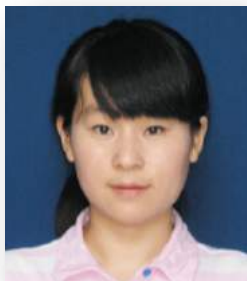


Liu Didi
Associate
Researcher

EHV



Qi Ting
Associate
Researcher
Flu
EAV



Ne Lei
Associate
Researcher

EIAV



Zhang Haili
Associate
Researcher

Flu



Zhang Zhenyu
Assistant
Researcher
Flu



Sun Like
Assistant
Researcher

Flu

Wang Xiaojun Bio

Education

Born in Inner Mongolia in 1974, researcher, doctoral supervisor

1992-1996

Bachelor of Veterinary Medicine, Inner Mongolia University for Nationalities

1996-1999

Master of Infectious Diseases and Preventive Veterinary Medicine, Graduate School of Chinese Academy of Agricultural Sciences,

2000-2003

Infectious Diseases and Preventive Veterinary Medicine, Graduate School of Chinese Academy of Agricultural Sciences, PhD

Work experience

1999-2005

Assistant Researcher, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences

2005-2010

Postdoctoral Fellow, Department of Microbiology and Molecular Genetics, Michigan State University, USA

2010-

Researcher, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences
Chief Expert of Equine Infectious Diseases and Lentivirus Research and Innovation Team

Research direction

Since 1999, he has been committed to the research of **lentivirus and equine infectious diseases**

Equine Infectious Disease and Lentiviral Disease Research Innovation Team

Research Direction:

Study on the interaction between virus and host innate immunity

Lentivirus, influenza virus

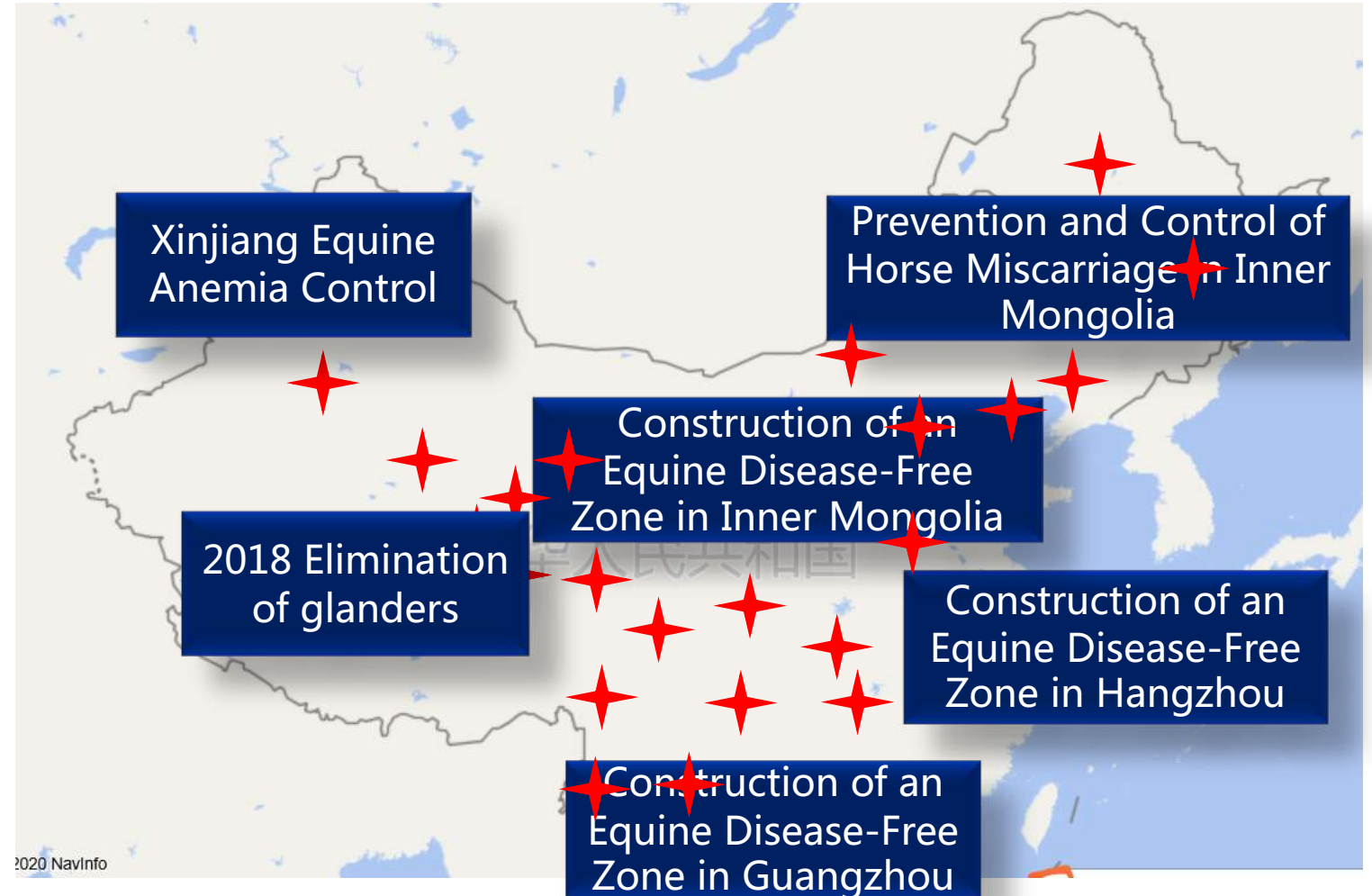
1. Study on the pathogenicity and immune mechanism of equine infectious diseases

Influenza virus, equine-borne anemia virus

2. Research on Epidemiology and Application Technology of Prevention and Control of Important Infectious Diseases of Equine

Laboratory Disease Surveillance and Technical Support

- National Equine Disease Surveillance and Control
- Construction of an epidemic-free area
- Elimination of glanders
- Intensive donkey farm service
- Pathogen identification and traceability

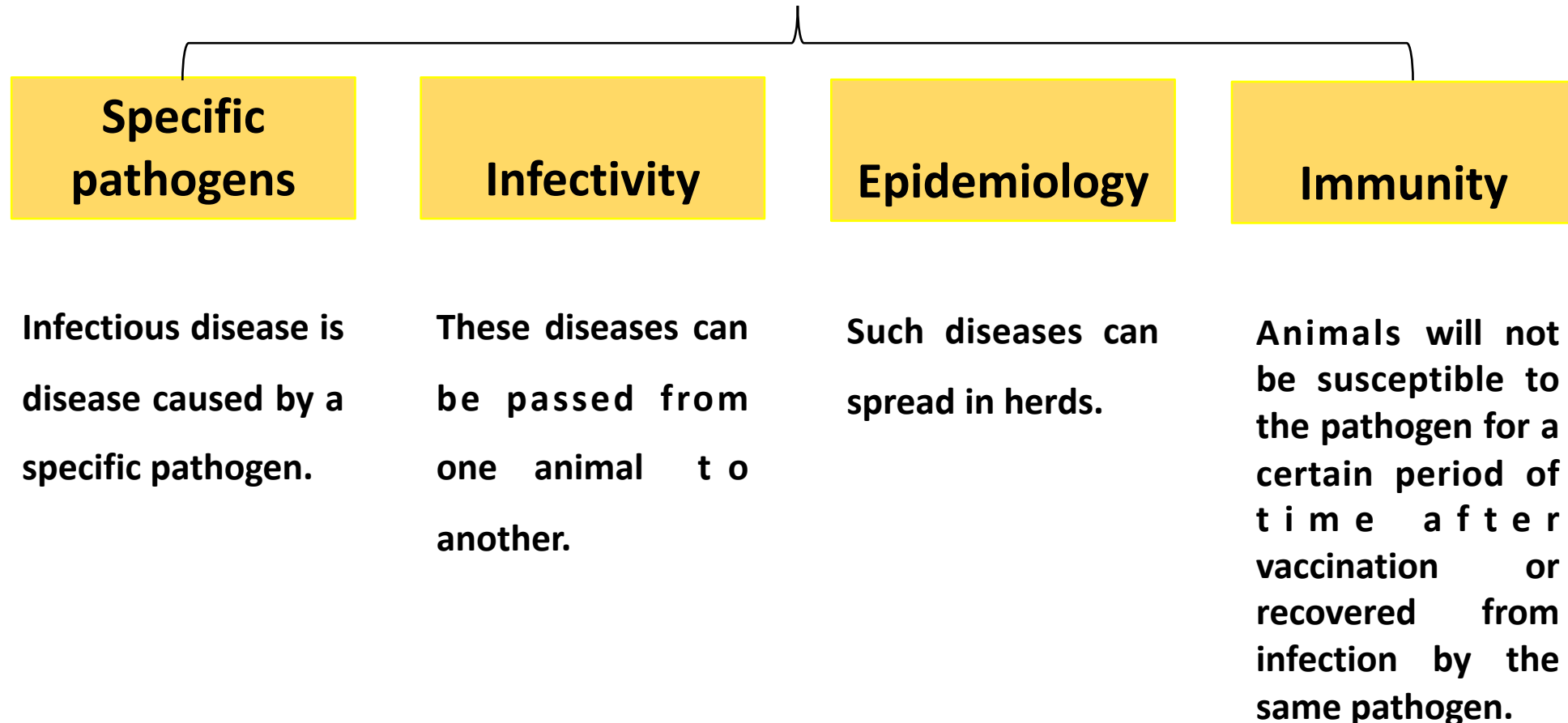


Classification of Equine Diseases

- © **Internal diseases**
- © **Surgical Diseases**
- © **Infectious Diseases**
- © . . .

Infectious Diseases

Fundamental Characters



Key Factors of Infectious Diseases

Infectious Agent

An animal capable of excreting pathogens (usually bacteria and virus) from the outside world.

Transmission Routes

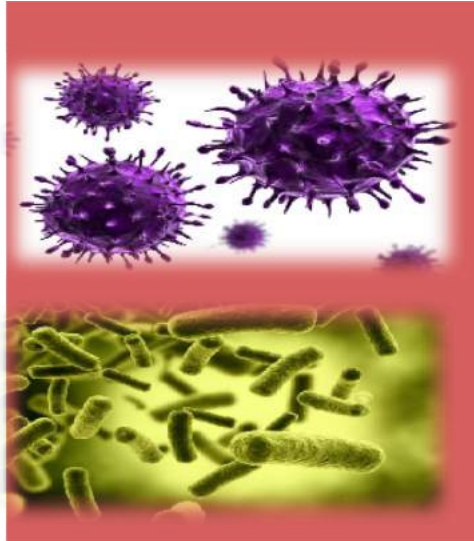
Air droplets;
Water, food;
Contact transmission;
Vector-borne transmission;
Soil-borne transmission ;
Blood-feeding insects;
Latrogenic transmission;
Vertical transmission;
Respiratory transmission;
Sexual transmission;
...

Susceptible Animals

Healthy animals with a high susceptibility to certain pathogens

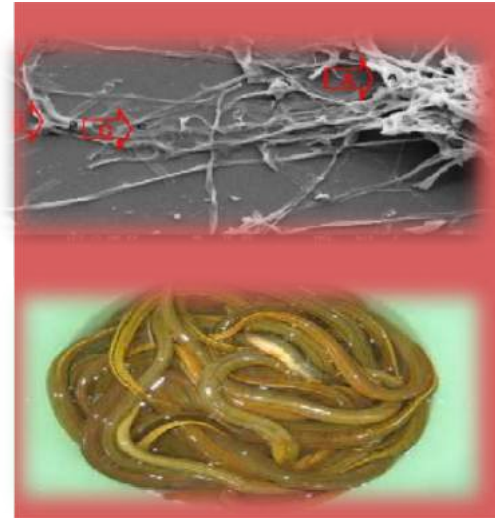
Pathogens of Equine Infectious Diseases

Virus



Bacteria

Fungus



Parasite

Infectivity

Virus

Bacteria

Fungus

Parasite

The speed of spread



Virus

Parasite

Bacteria

Fungus

The extent of epidemic



Equine viral diseases

- Equine infectious anemia
- Equine influenza
- Japanese encephalitis
- Equine viral arteritis
- Equine rhinopneumonitis
- African horse sickness
- Equine encephalomyelitis
- Equine rhinovirus infection
- Equine organic encephalopathy virus
- Parainfluenza type 3 virus infection
- Equine vesicular stomatitis
- Horsepox
- Equine adenovirus infection
- Equine papilloma
- Equine parvovirus infection
- Hendra disease
- Nipa disease
- West Nile
- Venezuelan equine encephalitis
- Borna disease
- Equine coronavirus infection

Equine bacterial and fungal diseases

- Equine adenitis
- Equine Salmonella
- Anthrax
- Epizootic lymphangitis
- Glanders
- Tetanus
- Dermatomycosis
- Equine contagious pleuropneumonia
- Contagious equine metritis
- Foal rhodococcus pneumonia
- Melioidosis
- Pasteurellosis
- Listeriosis
- Equine staphylococcosis
- Corynebacteriosis
- Botulism
- Malignant edema
- Equine necrobacillosis
- Actinomycosis
- Cryptococcosis
- Coccidiomycosis
- Ulcerative lymphangitis
- Penicilliosis marneffeii
- ...

Respiratory transmission

Cough + Runny nose → Air droplets

Respiratory transmission



Equine influenza

Equine rhinopneumonitis

Equine viral arteritis

Sexual transmission



Equine viral arteritis



Equine infectious anemia



Salmonella equine
abortion infection



Dourine

Equine rhinopneumonitis

Contact transmission



Glanders



Equine adenitis



Equine viral arteritis

Blood borne / insect transmission



Equine infectious anemia



Equine encephalomyelitis

Eastern equine encephalitis virus, EEEV;

Western equine encephalitis virus, WEEV ;

Venezuelan equine encephalomyelitis virus, VEEV

Clinical symptoms

Abortion



Equine viral arteritis

Equine Parvovirus
Infection

Equine herpesvirus 1 infection



Salmonella equine
abortion infection

Neurological symptoms



Equine encephalomyelitis

Eastern equine encephalitis virus, EEEV;
Western equine encephalitis virus, WEEV ;
Venezuelan equine encephalomyelitis virus, VEEV.



Nervous disorders, including
restlessness and shifting of weight
from leg to leg



Facial nerve numbness

Equine rhinopneumonitis



Tetanus

Dourine

Clinical signs

Equine influenza

Incubation period 3-7 days. The course of the disease is 5-30 days

- ✓ Fever
- ✓ Dry cough
- ✓ Running nose
- ✓ Depress, muscle soreness
- ✓ Reluctant to eat or drink
- ✓ Highly contagious
- ✓ Infect horses of any age



Differential diagnosis

**Equine contagious
bronchitis**

**Equine
rhinopneumonitis**

Equine rhinopneumonitis

Incubation period of several days – months. Course of disease 5-60 days

Caused by three distinct alpha herpes viruses, equine herpesvirus 1 (EHV-1), equine herpesvirus 4 (EHV-4), and equine herpesvirus 3 (EHV-3).

1. Congestion and clear nasal discharge
2. Mild to severe ataxia or paresis (slight or incomplete paralysis) of hind quarters
3. Fever for two to three days
4. Cough
5. Abortion after three to 12 weeks of exposure and most commonly in the 8th to 11th month of gestation.



Equine Infectious Anemia (Coggins' Disease)

Incubation period 10-40 days
Lifetime infection

1. High fever
2. Difficult breathing
3. Cardioacceleration, debility
4. Anemia.



Differential diagnosis

Surra (Istone trypanosomiasis)
Equine piroplasmosis
Equine leptospirosis
Nutritional anemia

Tetanus in horses

Incubation period 7-10 days
Course 4-6 weeks

1. Inability to open mouth to eat and drink
2. Eyes wide open and ears rigid
3. Stiffness and rigidity of the entire body
4. Extreme sensitivity to sounds, sights, and touch
5. Third eyelid closes uncontrollably
6. (One way to distinguish tetanus from other neurological diseases is to clap your hands and watch the third eyelid. It will close uncontrollably.)
7. Convulsions and death in 75 to 80 percent of cases.

Glanders

Incubation period several days – months.
Course of diseases varies.

Glanders is an infectious disease that is caused by the bacterium *Burkholderia mallei*. While people can get the disease, glanders is primarily a disease affecting horses. It also affects donkeys and mules and can be naturally contracted by other mammals such as goats, dogs, and cats.

- Chronic nasal discharge from one or both nostrils, with or without visible ulceration of the nasal septum;
- Chronic enlargement and hardening of the submaxillary lymph glands without outward discharge of pus;
- Presence of pustules and ulcers (farcy buds) on the skin of the hindlegs or other parts of the body.
- Nonclinical, or latent, cases are essentially pulmonary in type, and the lesions remain in a concealed state (occult) in the lungs as tubercle-like nodules and suppurating foci.



Salmonella equine abortion infection

1. Abortion often occurs in the second and third trimesters of pregnancy.
2. Before abortion, there are many signs, such as fever, breast swelling, vaginal bleeding with color liquid.
3. Most miscarriages are stillbirths and sepsis.

Incubation period several days – months, continuous infection



Strangles

- ✓ Quickly go off their feed
- ✓ Fever (39.4-41.1C)
- ✓ Wet cough with raspy, strained breathing
- ✓ Significant swelling between the lower jaw bone that may extend behind the cheekbone and along the sides of the face
- ✓ Produce copious greenish-yellow mucus

Incubation period several days - months
Course of the disease is more than 3 weeks



Factors to be considered for an infectious disease

Clinical

Affected Range

Morbidity

Contact history

Transmission
Characteristics

时

Disease and **Time Course**

空

Disease process and **Space**

Factors to be considered for an infectious disease

Clinical Laboratory

Affected Range

Morbidity

Contact history

Transmission
Characteristics

时

Disease and **Time** Course

空

Disease process and **Space**

Serological diagnosis

Pathogenic diagnosis

Clinical Diagnosis

Disease and **Time** Course

- ✓ Seasons
- ✓ Transmitting speed
- ✓ Recover/Death
- ✓ New member in the group
- ✓ Travel

Disease progress and **Space**

- Geographical environment
- Affected animals
- Wide animals
- Insect

Laboratory Diagnosis

Serological diagnosis

- ✓ **Serum**
- ✓ **Specific antibody**
- ✓ **Specific reagents /tests**
- ✓ **Known after infection**
- ✓ **Verification of infection/Immunity**

Pathogenic diagnosis

- **Blood samples, secreta, tissue**
- **Pathogen identification**
- **Specific reagents /tests**
- **In time**

Common equine disease serological testing methods (recommended)

疫病名称↵	检测项目↵	检测方法↵	确认方法↵
马传贫↵	抗体↵	cELISA↵	琼扩试验↵
马动脉炎↵	抗体↵	ELISA↵	中和试验↵
马流感↵	抗体↵	HI 试验↵	↵
马鼻疽↵	抗体↵	补反试验↵	↵
日本脑炎↵	抗体↵	ELISA↵	↵
马梨形虫病 努巴贝斯虫↵	抗体↵	ELISA↵	↵
马梨形虫病马泰勒虫↵	抗体↵	ELISA↵	↵
马媾疫↵	抗体↵	ELISA↵	中和试验↵
伊氏锥虫病↵	抗体↵	CATT 卡片凝集↵	↵
狂犬病↵	抗体↵	ELISA↵	↵
炭疽↵	抗体↵	沉淀反应↵	↵
马鼻肺炎 I 型疱疹病毒↵	抗体↵	ELISA↵	中和试验↵
马鼻肺炎 IV 型疱疹病毒↵	抗体↵	ELISA↵	中和试验↵
非洲马瘟↵	抗体↵	ELISA↵	↵
西尼罗河病↵	抗体↵	ELISA↵	↵

Nucleic acid detection methods (partial)

亨德拉病↵	核酸↵	qRT-PCR↵	测序↵
尼帕病毒病↵	核酸↵	qRT-PCR↵	测序↵
水泡性口炎印第安型↵	核酸↵	qRT-PCR↵	测序↵
水泡性口炎新泽西型↵	核酸↵	qRT-PCR↵	测序↵
马脑脊髓炎（东方）↵	核酸↵	qRT-PCR↵	测序↵
马脑脊髓炎（西方）↵	核酸↵	qRT-PCR↵	测序↵
马传染性子宫炎↵	核酸↵	qRT-PCR↵	测序↵
委内瑞拉马脑脊髓炎↵	核酸↵	qRT-PCR↵	测序↵
日本脑炎病毒↵	核酸↵	qRT-PCR↵	测序↵
西尼罗河病毒↵	核酸↵	qRT-PCR↵	测序↵
马流感病毒↵	核酸↵	qRT-PCR 和 RT-PCR↵	测序↵
马梨形虫病 努巴贝斯虫↵	核酸↵	巢式 PCR↵	测序↵
马梨形虫病马泰勒虫↵	核酸↵	巢式 PCR↵	测序↵
伊氏锥虫病↵	核酸↵	普通 PCR↵	测序↵

Professional Support

Knowledge and
expertise

Equipment and rapid
diagnosis

Specialized laboratory

Key laboratory diagnostic technologies and products

马属动物（马、驴）主要传染病检测试剂盒明细表

编号	马传染病病名	试剂盒名称
1	马传染性贫血	马传染性贫血病毒 cELISA 抗体检测试剂盒
2		马传染性贫血琼脂试验抗原、阳性与阴性血清试剂盒
3		马传染性贫血抗体胶体金检测卡
4		马传贫病毒荧光 PCR 检测试剂盒-A 版（探针法）
5		马传贫病毒荧光 PCR 检测试剂盒-B 版（探针法）
6	马流行性感冒	H3N8 亚型马流感 HI 试验抗原和阴阳性抗体
7		马流感竞争 ELISA 抗体检测试剂盒
8		马流感病毒一步法 RT-PCR 检测试剂盒
9		马流感病毒一步法荧光 RT-PCR 检测试剂盒（探针法）
10		马流感病毒一步法等温快速扩增试剂盒（重组酶法）
11		马流感病毒 AC-ELISA 抗原检测试剂盒
12	马动脉炎	马动脉炎病毒一步法荧光 RT-PCR 检测试剂盒（探针法）
13		马动脉炎病毒一步等温快速扩增试剂盒（重组酶法）
14	马鼻肺炎	I 型马疱疹病毒荧光 PCR 检测试剂盒（探针法）
15		IV 型马疱疹病毒荧光 PCR 检测试剂盒（探针法）
16		马疱疹病毒 I/IV 型单管双重荧光 PCR 检测试剂盒（探针法）
17	日本脑炎	日本脑炎 RT-PCR 检测试剂盒
18		日本脑炎 RT-LAMP 检测试剂盒（可视化）
19	亨德拉尼帕病毒属	亨德拉尼帕病毒属一步法荧光 RT-PCR 检测试剂盒（探针法）
20	非洲马瘟	非洲马瘟病毒一步法荧光 RT-PCR 检测试剂盒（探针法）
21		非洲马瘟病毒一步法等温快速扩增试剂盒（重组酶法）

Equine disease detection kit

22	马梨形虫病	马梨形虫（马泰勒虫）竞争 ELISA 抗体检测试剂盒	
23		马梨形虫（鸢巴贝斯虫）竞争 ELISA 抗体检测试剂盒	
24		马梨形虫（马泰勒虫）抗体胶体金检测卡	
25		马梨形虫（鸢巴贝斯虫）抗体胶体金检测卡	
26		马梨形虫（马泰勒虫和鸢巴贝斯虫）PCR 检测试剂盒	
27		马梨形虫（马泰勒虫）荧光 PCR 检测试剂盒	
28		马梨形虫（鸢巴贝斯虫）荧光 PCR 试剂盒	
29		马梨形虫（巴贝斯虫和鸢巴贝斯虫）双重荧光 PCR 试剂盒	
30		马流产沙门氏菌病	马流产沙门氏菌间接 ELISA 抗体检测试剂盒
31			马流产沙门氏菌 cELISA 抗体检测试剂盒
33	马流产沙门氏菌抗体胶体金检测卡		
34	马流产沙门氏菌 PCR 检测试剂盒		
35	马流产沙门氏菌荧光 PCR 检测试剂盒（探针法）		
36	马腺疫	马、驴腺疫 PCR 检测试剂盒	
37	伊氏锥虫病	伊氏锥虫 PCR 检测试剂盒	

Laboratory diagnosis of equine infectious anemia

2.4 实验室诊断

2.4.1 马传贫琼脂扩散试验 (AGID) (见附件)。

2.4.2 马传贫酶联免疫吸附试验 (ELISA) (见附件)。

2.4.3 马传贫病原分离鉴定 (见附件)。

2.4.4 结果判定

具备马传贫流行特点、临床症状、病理变化, 可做出初步诊断;

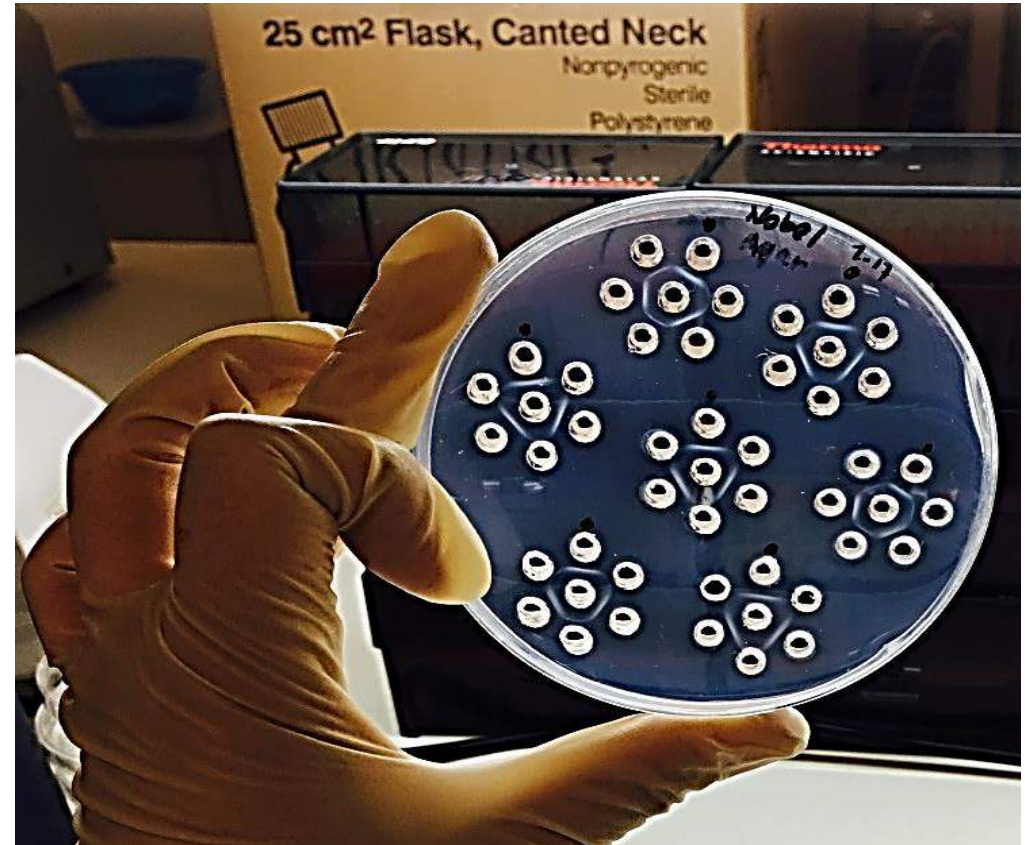
2.4.1 或 2.4.2 或 2.4.3 结果阳性, 即可确诊。

New Generation of AGID kit by HVRI

Precipitation line can appear in 12 hours, which is better than similar international kits



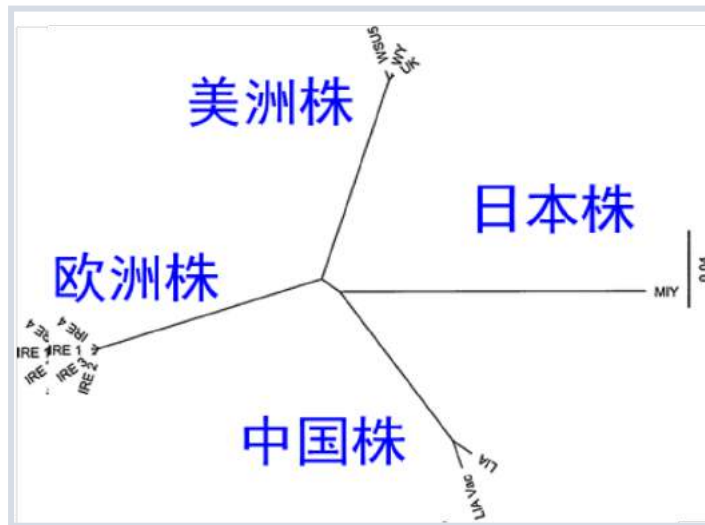
24 hours



48 hours

Key technical support

Need for more accurate, sensitive, and faster diagnostic methods



Large strain variation

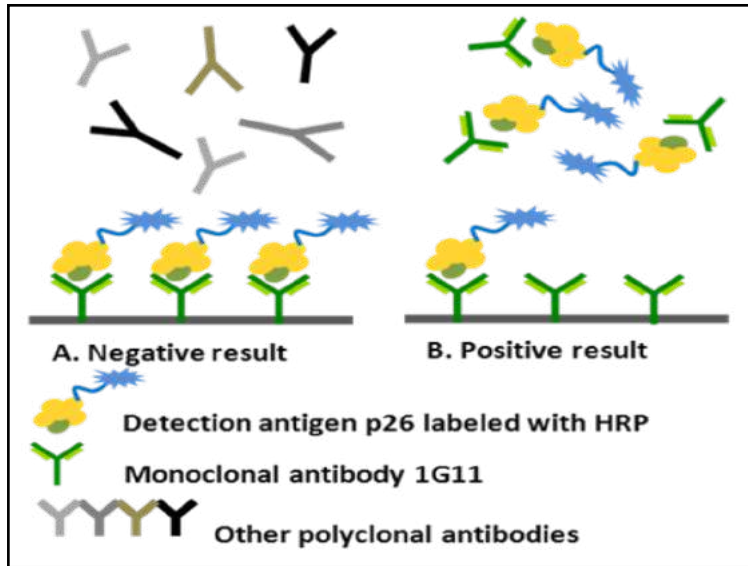


The current international standard – AGID is seriously outdated



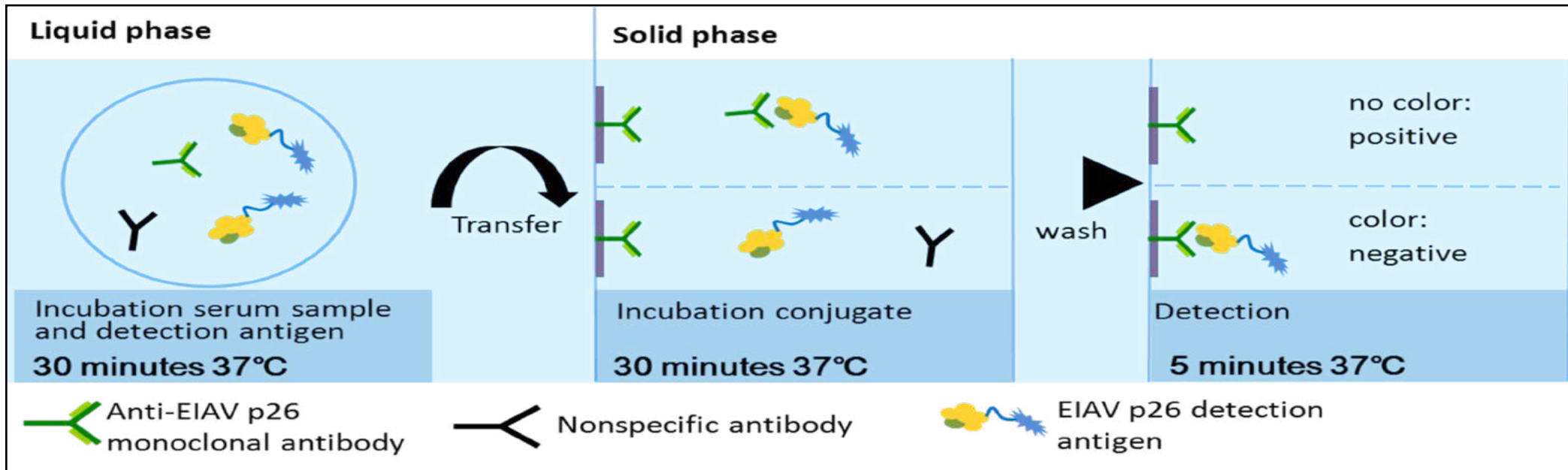
There is an urgent need for **sensitive, accurate, high-throughput**, and key detection technologies

Successfully established a cELISA kit for horse-borne anemia



Advantage:

- Fast
- High throughput
- Good specificity
- High sensitivity



Successfully developed cELISA rapid detection kit

Equine infectious
anemia antibody
cELISA detection kit

PATENT,
ZL201410239152.7
*Appl Microbiol
Biotechnol.* 2014



Internationally leading

Accurate: no false positives
Sensitive: 8 times higher than
AGID

Fast: 1.5 hours to complete
High throughput: 500
samples per person

International verification :

- Spain
- National Institute of Virology
of Argentina
- Hong Kong Agriculture and
Fisheries Department
- Dubai OIE Equine Disease
Reference Laboratory




Test **500 samples**

AGID required: 7
days

cELISA takes: **2
hours**

Detection rate
increased by **132%**

Standard Reference Positive Serum Test

Method	Kit	VMRD Anti-EIAV		
		Strong	Medium	Weak
cELISA	HVRI	+	+	+
	Inhibition Rate	99.39%	88.42%	67.45%
AGID	IDEXX	-	-	-
cELISA	IDEXX	-	-	-
Western blot	1000 dilution of serum			

International Comparison Proves Advanced Technology

No.	Sample ID	Store No.	Harbin-ELISA	AGID	Idexx-ELISA	Eradikit-ELISA
158	SE 15/20	S135-58	Negative	Negative	Negative	Positive
159	SE 17/20	S136-9	Negative	Negative	Negative	Positive
160	SE 91.2/20	S09-39	Negative	Negative	Negative	Doubtful
161	SE 135.1/20	S06-2	Negative	Negative	Negative	Doubtful
162	SE 211.14/20	S08-58	Negative	Negative	Negative	Doubtful
163	SE 284.2/20	S10-45	Negative	Negative	Negative	Positive
164	Muneca	-	Positive 98.1%	Positive	Positive	Positive
165	EQC 17/7839	-	Positive 99.1%	Strong Positive	Positive	Positive
166	EQC 17/7840	-	Negative	Negative	Negative	Negative
167	EQC 17/7841	-	Positive 99.4%	Positive	Positive	Positive
168	IdVet Ref.sera Neat	-	Positive 98.6%	Positive	Positive	Positive
169	IdVet Ref.sera 1:4	-	Positive 90.5%	Negative	Negative	Positive

True Negatives: 163 (equine samples); 1 (EQC Negative)

Specificity

Harbin ELISA	100%
AGID	100%
Idexx ELISA	100%
Eradikit ELISA	77.30%

True Positives: 1 (Field case); 2 EQC (positive); 2 (Reference sera)

Sensitivity

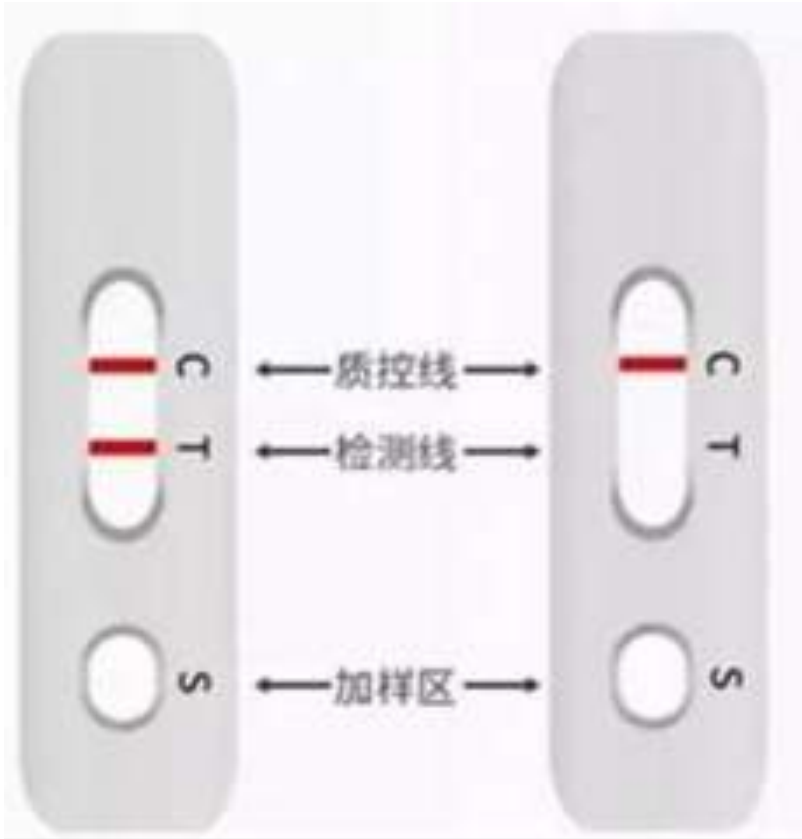
Harbin ELISA	100%
AGID	83.30%
Idexx ELISA	83.30%
Eradikit ELISA	100%



CENTRAL VETERINARY RESEARCH LABORATORY, DUBAI, UAE

The comparison of the OIE reference laboratories of Argentina, the Hong Kong Agriculture and Fisheries Department, and the World Organization for Animal Health in Dubai proved that the indicators of this method are better than those of similar international products.

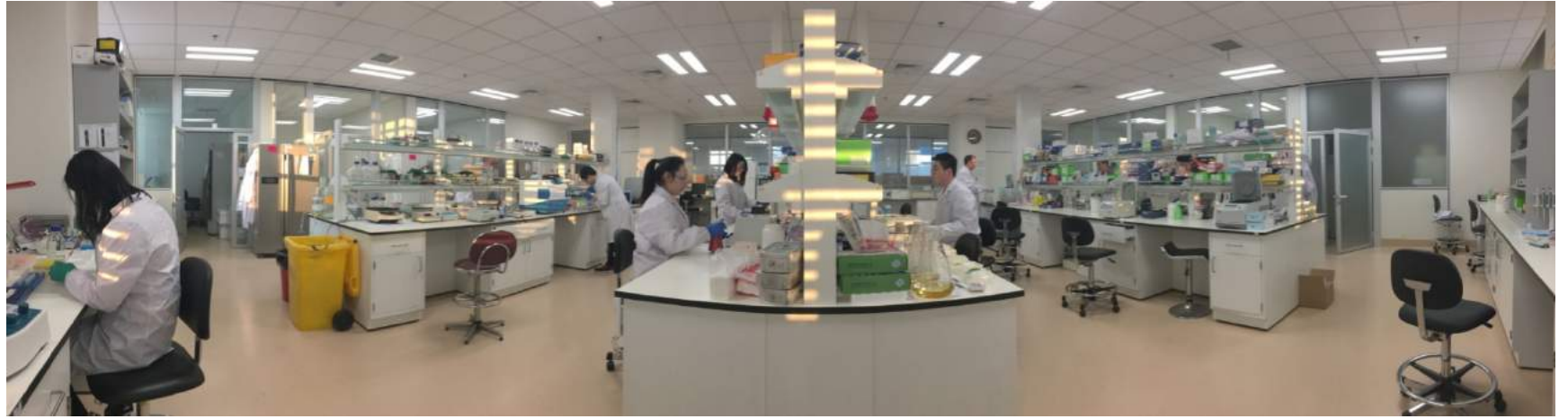
Rapid Colloidal Gold Test Strip



- **Accuracy:** equivalent to the AGID gold standard
- **Sensitivity:** equivalent to cELISA
- **Fast:** Results in 10 minutes
- **A drop of blood test**
- **No training required**



Specialized Laboratory



Harbin Veterinary Research Institute

- State Key Laboratory of Veterinary Biotechnology
- National Glanders Reference Laboratory
- National Equine Infectious Anemia Reference Laboratory
- OIE Reference Laboratory for Equine Infectious Anemia



Summary

**Discover clinical
sign in time**

Rapid diagnosis

**Segregation once
infectious disease
happened**

**Strengthen the
prevention and control
of infectious diseases**

**Knowledge and
expertise**

**Equipment and rapid
diagnosis**

Specialized laboratory

Thank you for your attention !

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