World Organisation for Animal Health
Founded in 1924

The advance in FMD Diagnosis by LVRI, China

He Jijun

LVRI,CAAS

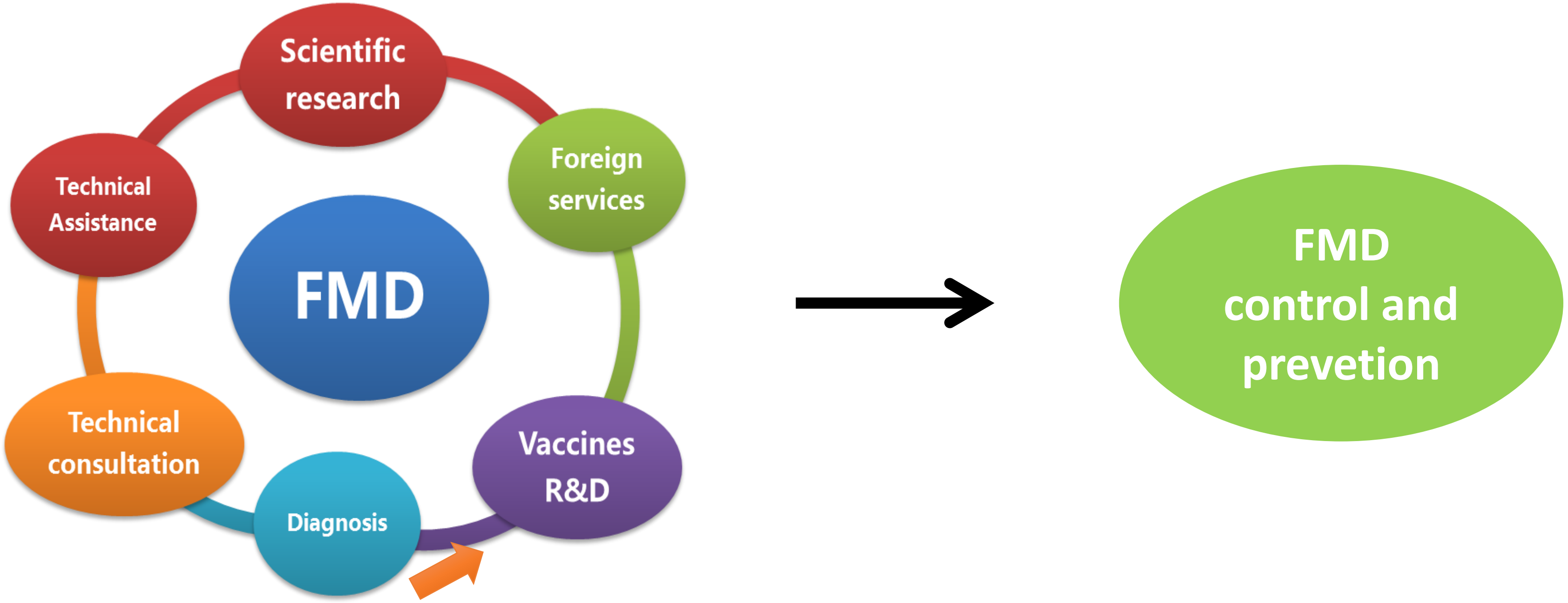
WOAH/national RL for FMD

LVRI

Outline

1. FMD diagnostic technology system, products and quality assurance at LVRI
2. Recent advance in FMD Diagnosis at LVRI

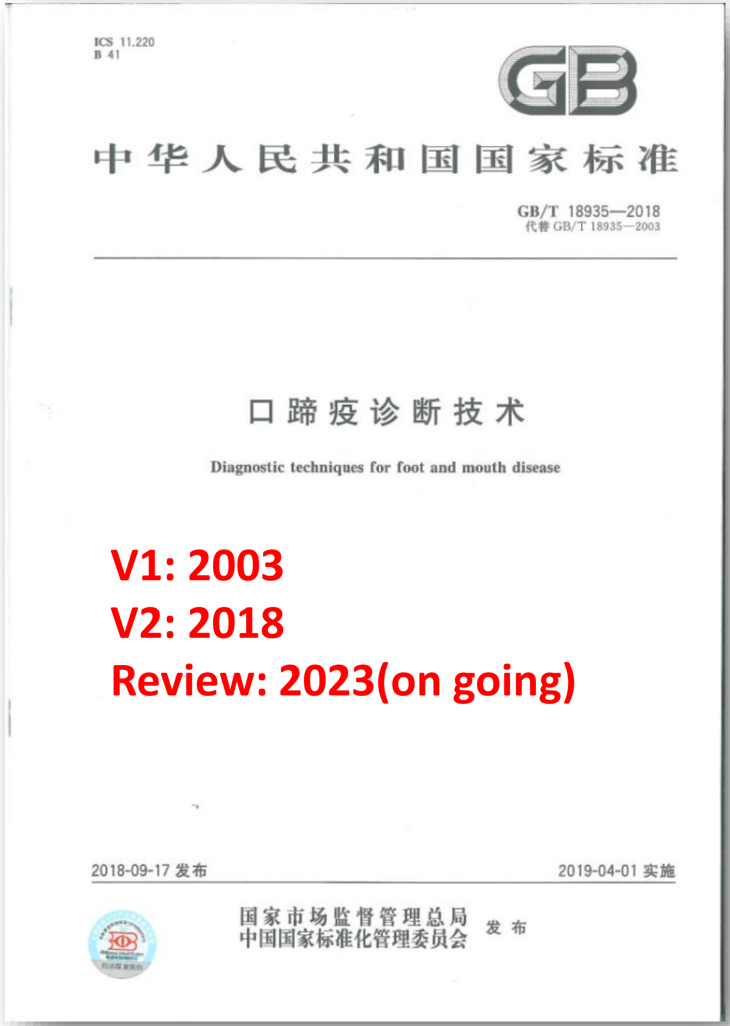
Roles of national FMD RL



1.1 FMD diagnostic methods recommended by WOAH and nation

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection and identification of the agent[a]						
Virus isolation	–	+	+++	+++	–	–
Antigen detection ELISA	–	–	+++	+++	–	–
CFT	–	–	+	+	–	–
LFD	–	–	+++	+++	–	–
Real-time RT-PCR	+	+	+++	+++	+	–
RT-PCR	+	+	+++	+++	+	–
Detection of immune response						
NSP Ab ELISA	+++	++	+++	+++	+++	–
SP Ab ELISA[b]	++	++	+++	+++	++	+++
VNT[b]	++	++	+++	+++	++	+++
AGID[b]	+	+	+	+	+	–

WOAH *Terrestrial Manual, 3.1.8*


ICS 11.220
B 41

GB

中华人民共和国国家标准

GB/T 18935—2018
代替 GB/T 18935—2003

口蹄疫诊断技术

Diagnostic techniques for foot and mouth disease

2018-09-17 发布 2019-04-01 实施

国家市场监督管理总局
中国国家标准化管理委员会 发布

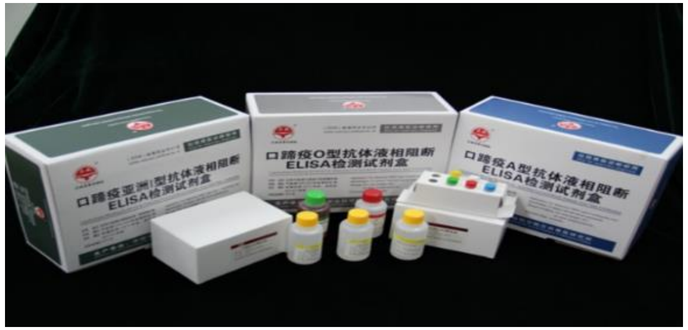
V1: 2003
V2: 2018
Review: 2023(on going)

National standard of diagnostic techniques for FMD

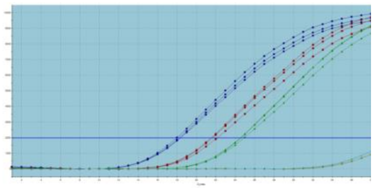
1.2 FMD diagnostic products in LVRI

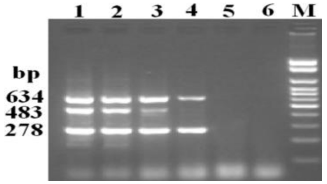
R&d time	Kit name	New veterinary drug ID
2006.11.08	FMDV mutiplex RT-PCR kit	(2006)新兽药证字38号
2006.11.08	FMDV（O、A、C、Asia1）typing RT-PCR kit	(2006)新兽药证字39号
2006.11.08	FMD Asia1 antibody LPBE kit	(2006)新兽药证字41号
2006.11.08	FMD O antibody LPBE kit	(2006)新兽药证字42号
2006.11.29	FMDV NSP 3ABC indirect ELISA	(2006)新兽药证字69号
2015.06.24	FMDV NSP 2C3AB antibody LFD	(2015)新兽药证字23号
2018.12.10	FMD O antibody LFD	(2018)新兽药证字66号
2020.10.21	FMDV O type C-ELISA antibody kit	(2020)新兽药证字52号
2021.03.18	FMDV A type antibody LFD	(2021)新兽药证字13号
2021.07.16	FMD NSP 3ABC block ELISA kit	(2021)新兽药证字46号
10 FMD diagnostic kits Registered a new veterinary drug certificate		

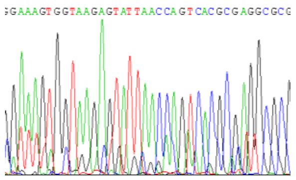
Establish test methods system according to WOAH standards, and Explore a series of diagnostic products (kits)



1.3 Procedure of FMDV diagnosis at LVRI



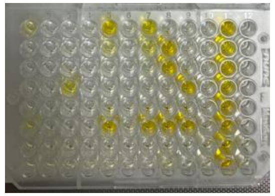




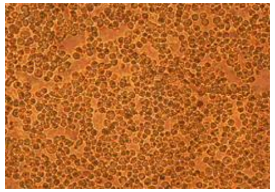
Molecular detection(RT-qPCR, RT-PCR, sequencing)

Clinical samples

Serotyping (Capture ELISA)



Virus Isolation (BHK21, BTY)



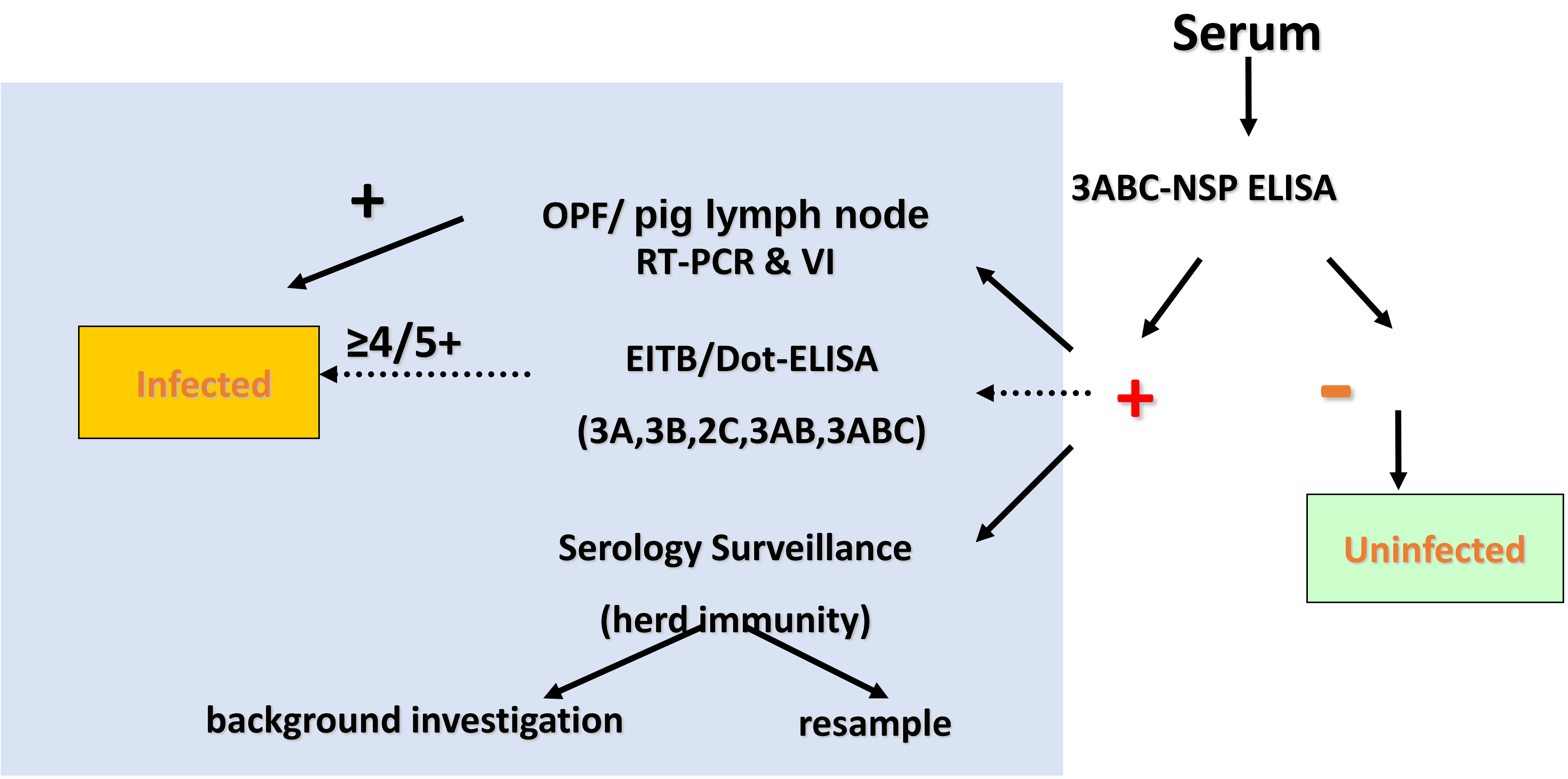
fresh ES, Vesicular fluid…

But, frequently,

"bad samples"

- Discovery lately
- Reporting lately
- Delayed sampling
- Improper Transporting
- Vaccinated animals
- Temperature
- Long time preservation
- ……

Differential diagnosis for FMD vaccinated or infected antibody



1.4 Quality assurance of FMDV diagnosis in LVRI

Laboratory Quality Management System
ISO/IEC 17025
Accredited in 2017, reviewed in 2023

Tests accredited	Accreditation body
LPB ELISA for FMDV antibody detection	CNAS
SPC ELISA for FMDV antibody detection	CNAS
ELISA for FMDV NSP antibody detection	CNAS
virus neutralization test (VNT)	CNAS
virus isolation(VI)	CNAS
FMDV Antigen detection ELISA	CNAS
RT-PCR for FMDV	CNAS
Real-time RT-PCR for FMDV	CNAS
FMDV 1D Gene sequencing	CNAS


ilac-MRA CNAS

China National Accreditation Service for Conformity Assessment

LABORATORY ACCREDITATION CERTIFICATE

(Registration No. CNAS L10176)

Center for Diagnosis and Testing, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences

No.1, Xujiaping, Yanchangbu, Chengguan Distruct, Lanzhou, China

is accredited in accordance with ISO/IEC 17025: 2005 General Requirements for the Competence of Testing and Calibration Laboratories(CNAS-CL01 Accreditation Criteria for the Competence of Testing and Calibration Laboratories) for the competence to undertake the service described in the schedule attached to this certificate.

The scope of accreditation is detailed in the attached schedule bearing the same registration number as above. The schedule form an integral part of this certificate.

Date of Issue：2017-07-27
Date of Expiry：2023-07-26
Date of Initial Accreditation：2017-07-27

Signed on behalf of China National Accreditation Service for Conformity Assessment

China National Accreditation Service for Conformity Assessment(CNAS) is authorized by Certification and Accreditation Administration of the People’s Republic of China (CNCA) to operate the national accreditation schemes for conformity assessment. CNAS is a signatory of the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement (ILAC MRA) and the Asia Pacific Laboratory Accreditation Cooperation Mutual Recognition Arrangement (APLAC MRA).

The validity of the certificate can be checked on CNAS website at http://www.cnas.org.cn/english/findanaccreditedbody/index.shtml

2. Recent advance in FMD Diagnosis

2.1 Development of antibodies detection of Competitive ELISA (SPCE)

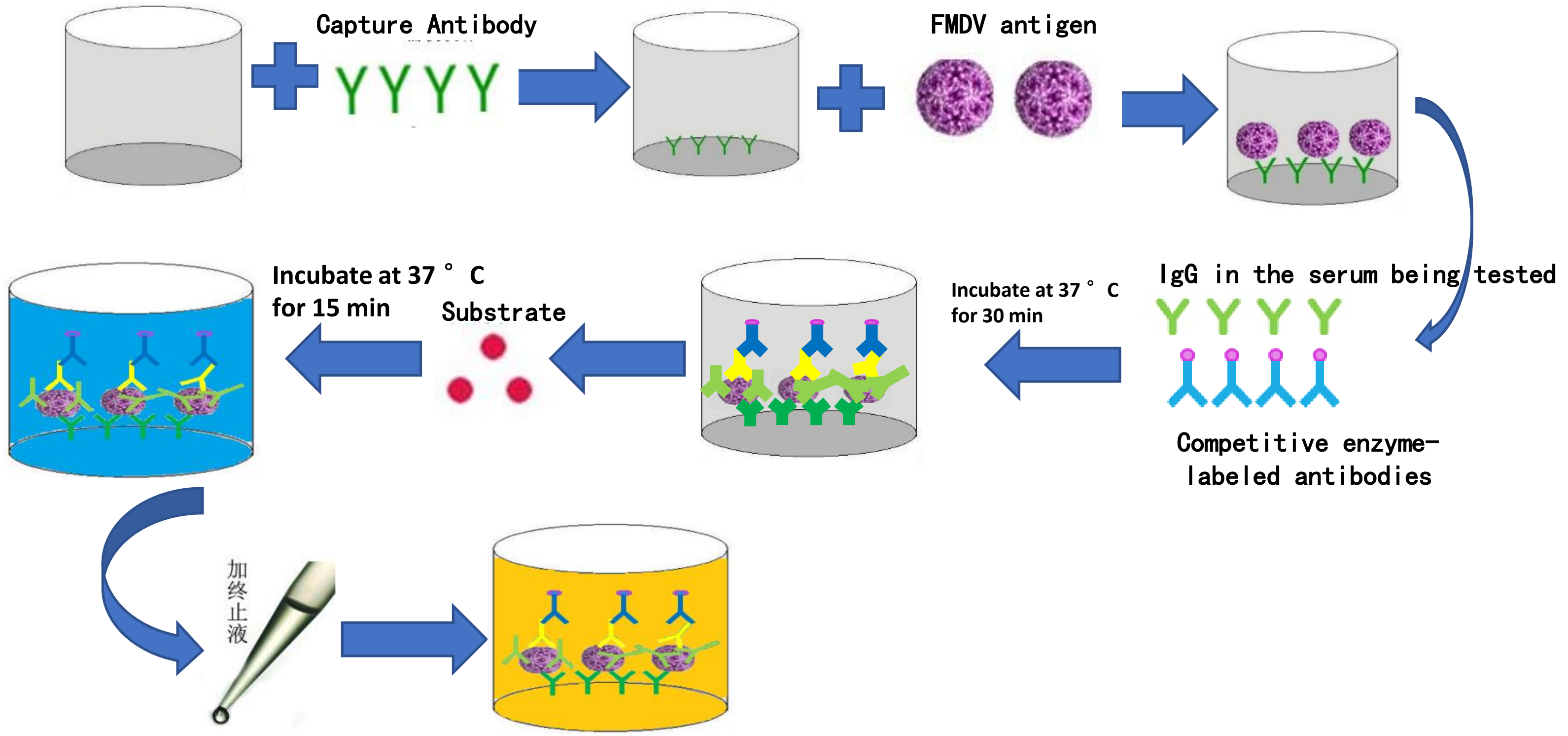
2.2 Technology Platform of LFIA

2.3 CLIA for detection of antibodies against FMDV SP and NSP

2.4 Preparation of diagnostic methods for SAT2

2.5 other joint detection methods

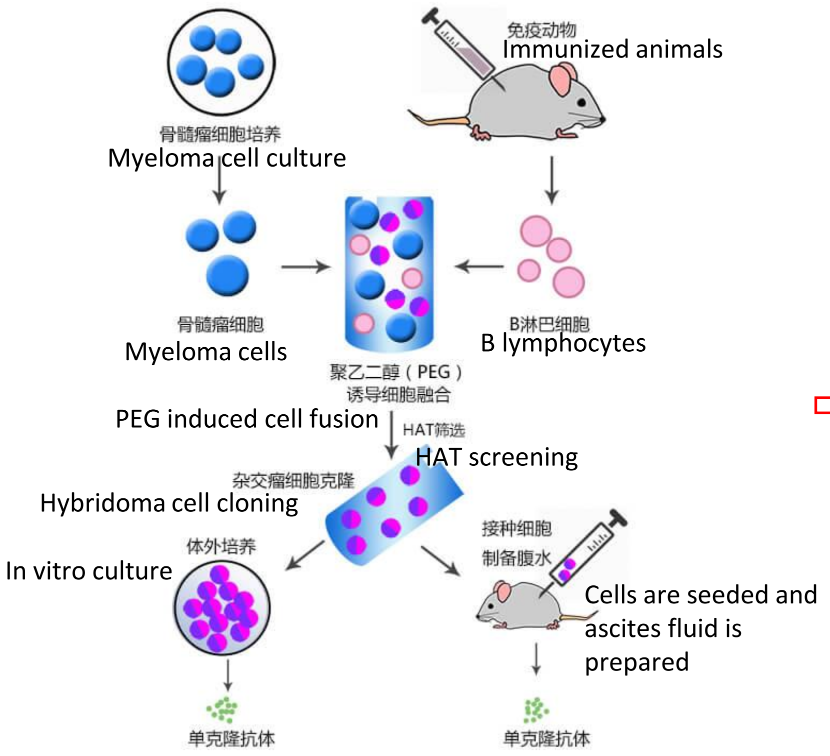
2.1 Antibodies Detection of Competitive ELISA （SPCE）



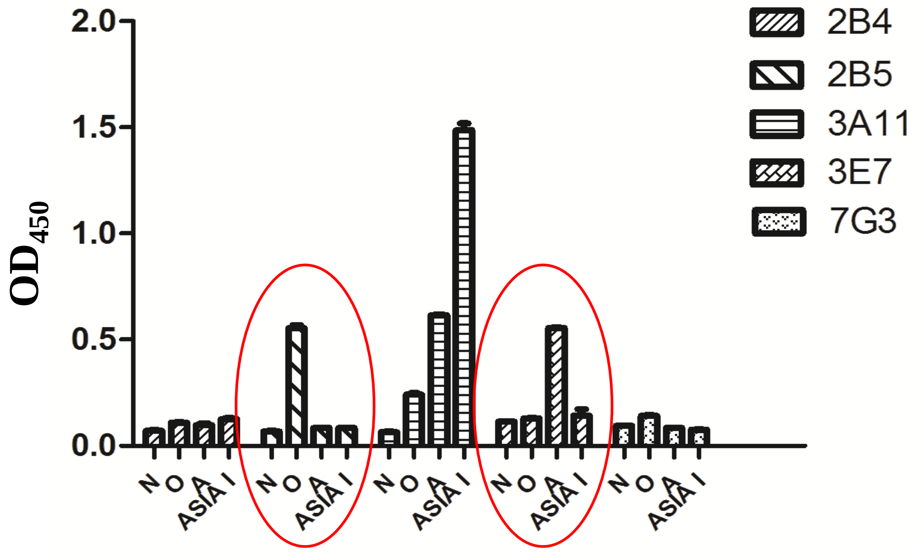
type O, type A

Contact： 田宏 tianhong@caas.cn

Screening for the specific antibodies of FMDV serotype O、A



Monoclonal antibodies



Traditional hybridoma technology was used to successfully screen the serotype-specific antibodies against FMDV type O and A

The features of FMDV antibodies detection of SPCE

- The operation is simple, reaction is only in one-step process, and
- The reaction time is about 42 min,
- Both serotype and titer could be determined
  - determine the serotype, without cross-reaction, has high conformity with VNT.
  - accurately determine the titer to evaluate the efficacy of the vaccines.

Titer	<8	8	11	11	<8	<8	<8	<8	<8	<8		
	P3687	P3673	P3661	P3679	P3682	P3642	P3638	P3672	P3655	P3668	Positive	Negative
	1	2	3	4	5	6	7	8	9	10	11	12
1：8	1.255	0.914	0.796	0.896	1.198	1.192	1.187	1.238	1.067	1.208	0.188	1.279
1：16	1.487	1.225	1.138	1.334	1.431	1.466	1.374	1.362	1.411	1.31	0.208	1.438
1：32	1.5	1.28	1.165	1.31	1.261	1.237	1.361	1.33	1.331	1.375	0.295	1.363
1：64	1.535	1.312	1.255	1.377	1.38	1.37	1.404	1.369	1.431	1.422	0.481	1.502
1：8	1.125	1.054	0.986	1.056	1.019	1.06	1.086	1.088	1.024	0.966	0.628	1.486
1：16	1.4	1.308	1.218	1.347	1.286	1.272	1.321	1.256	1.245	1.157	0.81	1.532
1：32	1.345	1.329	1.33	1.369	1.328	1.279	1.313	1.323	1.314	1.277	1.013	1.514
1：64	1.51	1.433	1.471	1.595	1.638	1.37	1.505	1.382	1.489	1.47	1.22	1.615
	P3684	P3659	P3683	P3693	P3678	P3624	P3631	P3690	P3686	P3674	360	1.53675
Titer	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8		0.922

	174	382	459	139	320	2	9	471	12	P0722	Positive control		
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.212	0.119	0.194	0.173	0.105	0.2	0.106	0.217	0.13	0.293	0.521	2.186	450
B	0.379	0.411	0.535	0.403	0.27	0.354	0.197	0.647	0.296	0.371	0.266	2.311	450
C	0.437	0.433	0.878	0.659	0.289	0.247	0.233	0.817	0.591	0.587	0.328	2.307	450
D	0.857	0.586	1.087	0.963	0.283	0.358	0.337	0.993	0.709	0.954	0.511	2.324	450
E	0.977	0.805	1.391	1.351	0.357	0.462	0.646	1.359	0.997	1.366	0.808	2.242	450
F	1.387	1.247	1.708	1.646	0.637	0.683	0.922	1.645	1.383	1.627	1.228	2.319	450
G	1.682	1.594	1.835	1.879	0.985	1.006	1.293	1.866	1.707	1.819	1.593	2.309	450
H	2.098	1.944	2.085	2.161	1.456	1.438	1.781	2.079	2.164	2.075	1.931	2.442	450
Titer	360	360	128	180	720	720	720	180	256	180	360		

2.2 Technology Platform of LFIA

- 8 kinds of livestock diseases diagnostic test-strip
- 3 obtained the Veterinary Drug Product Approval Number

2008

Colloidal Gold LFIA

Quick and Easy

15 minutes

2017

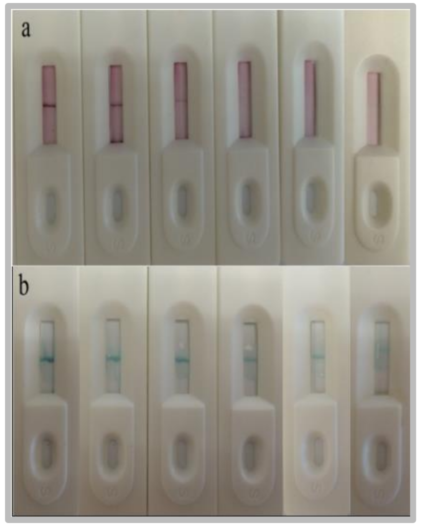
Peroxidase-like Nanoparticles LFIA

First Application

2021-2023

Fluorescence Microspheres LFIA

Digitization Accurate and Sensitive



中华人民共和国

新兽药注册证书

证号：（2021）新兽药证字 13 号

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：

中华人民共和国

新兽药注册证书

证号：（2018）新兽药证字 44 号

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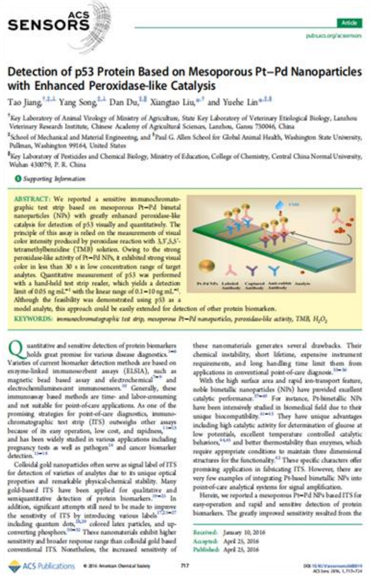
发证日期：



ACS SENSORS

Article

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Detection of p53 Protein Based on Mesoporous Pt–Pd Nanoparticles with Enhanced Peroxidase-like Catalysis

Tao Jiang, Yang Song, Dan Du, Xiangtao Liu,* and Yuehe Lin*

Key Laboratory of Animal Virology of Ministry of Agriculture, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu 730046, China

School of Mechanical and Material Engineering, and Paul G. Allen School for Global Animal Health, Washington State University, Pullman, Washington 99164, United States

Key Laboratory of Pesticides and Chemical Biology, Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, P. R. China

Supporting Information

ABSTRACT: We reported a sensitive immunochromatographic test strip based on mesoporous Pt–Pd bimetal nanoparticles (NPs) with greatly enhanced peroxidase-like catalysis for detection of p53 visually and quantitatively. The principle of this assay is relied on the measurements of visual color intensity produced by peroxidase reaction with 3,3′,5,5′-tetramethylbenzidine (TMB) solution. Owing to the strong

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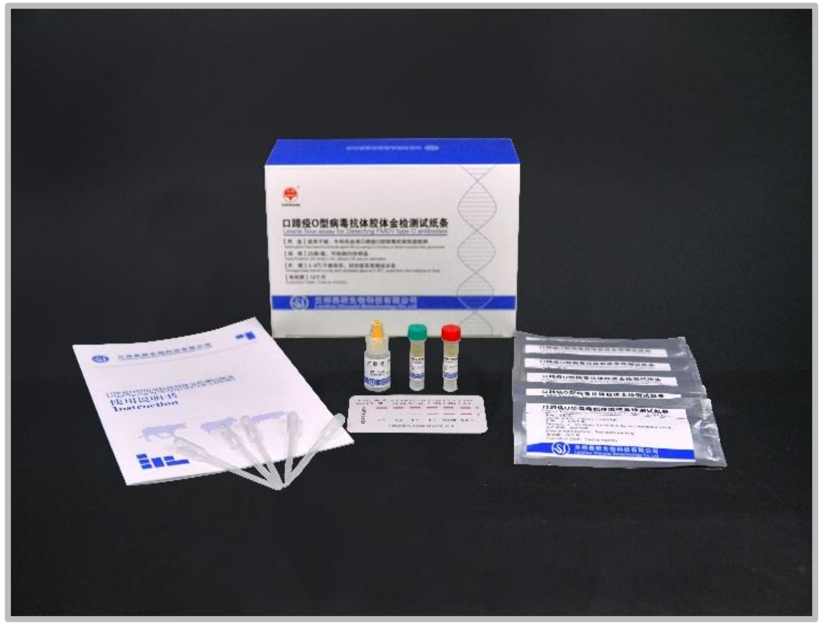
Products: technological Innovation of LFIA

The list of LFIA kits

1	Lateral Flow Assay Kit for Detecting Antibodies against FMDV Type O	New Veterinary Drug Registration Certificate ：no.（2018） New Veterinary Drug Certificate no.66 ；Veterinary Drug Product Approval Number ：Veterinary Medicine 280178855 ；25 strips/kit; to detect 25 sera samples; The results of 85% of samples were recorded to reach the standard recommended by the OIE LPB-ELISA method; This operation-friendly is suitable for on-site testing,the results of which can be obtained within 10 minutes.
2	Lateral Flow Assay Kit for Detecting Antibodies against FMDV Type A	New Veterinary Drug Registration Certificate ：no.（2021） New Veterinary Drug Certificate no.13 ；25 strips/kit; to detect 25 sera samples; The results of 85% of samples were recorded to reach the standard recommended by the OIE LPB-ELISA method; This operation-friendly is suitable for on-site testing,the results of which can be obtained within 10 minutes.
3	Lateral Flow Assay Kit for Detecting Antibodies against FMDV Type Asia-I	25 strips/kit; to detect 25 sera samples; The results of 85% of samples were recorded to reach the standard recommended by the OIE LPB-ELISA method; This operation-friendly is suitable for on-site testing,the results of which can be obtained within 10 minutes.
4	Lateral Flow Assay Kit for Detecting Non-structural Protein 2C3AB Antibodies against FMD	New Veterinary Drug Registration Certificate ：no.（2015） New Veterinary Drug Certificate no.23 ；Veterinary Drug Product Approval Number ：Veterinary Medicine 280178215 ；20 strips/kit; to detect 20 sera samples; The results of 90.1% of samples were recorded to reach the standard of the OIE recommended 3ABC-ELISA method; This operation-friendly and equipment-free product can be applied to on-site testing.

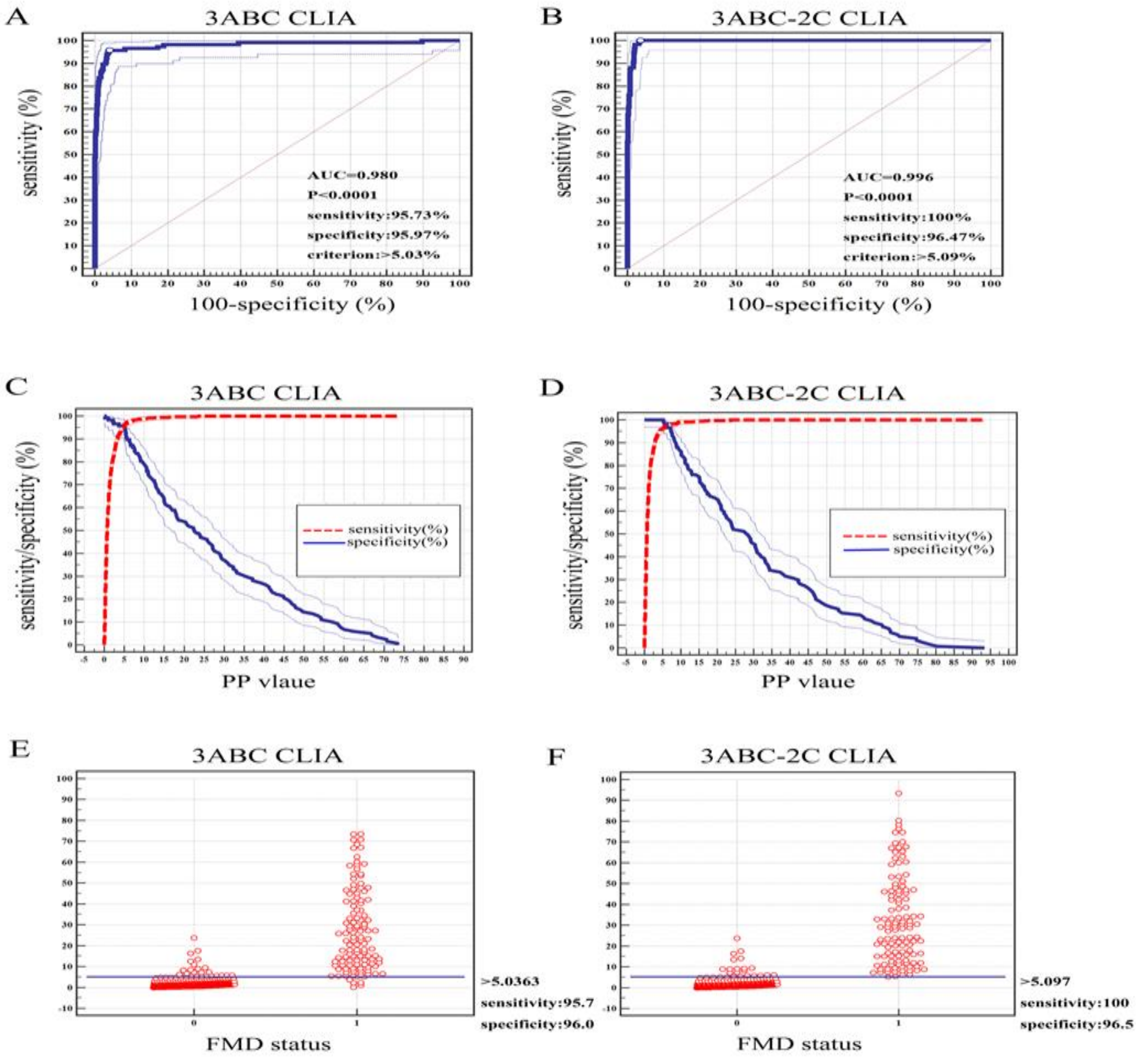


兰州兽研生物科技有限公司
Lanzhou Shouyan Biotechnology Co. , Ltd

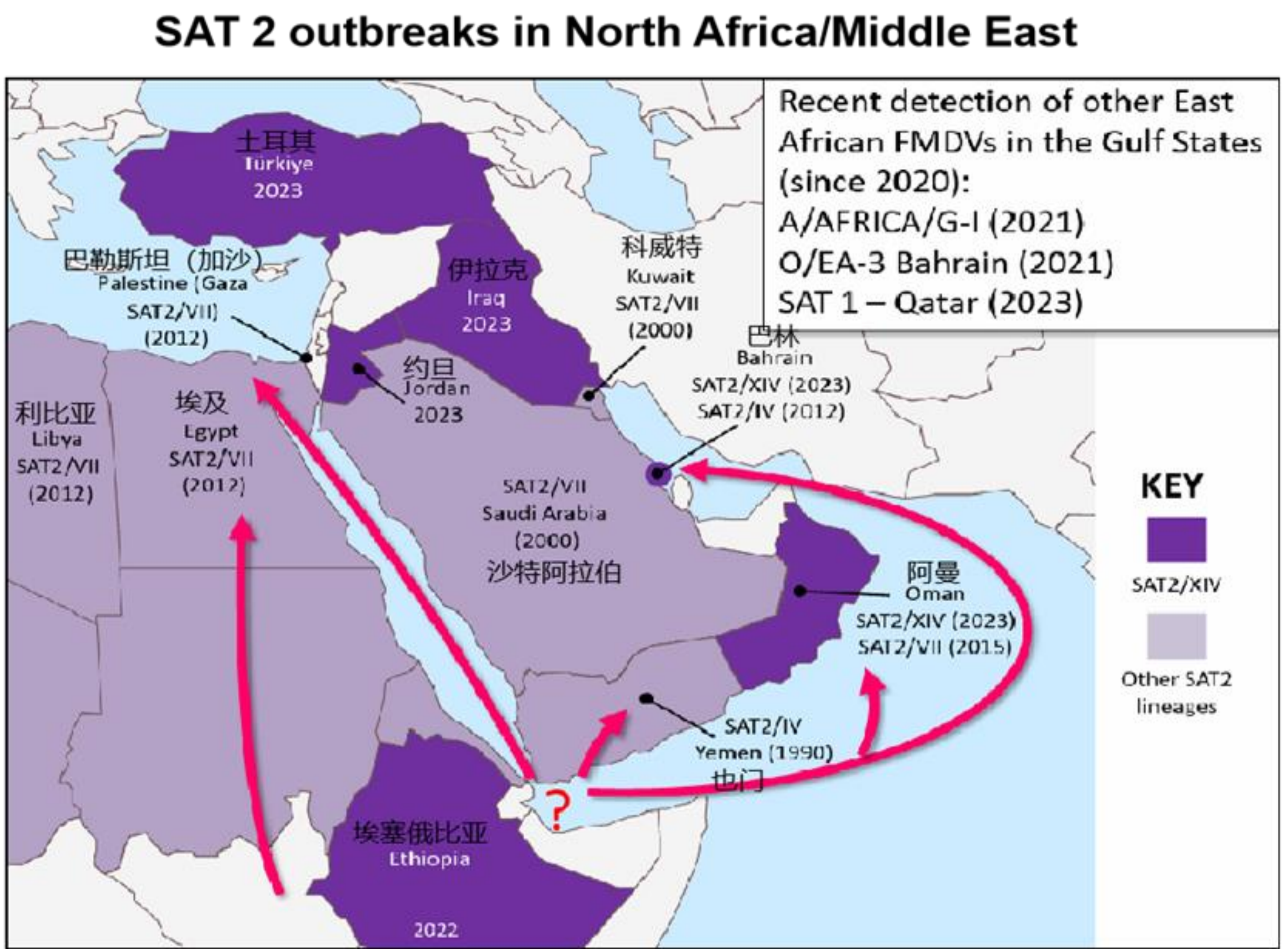
2.3 CLIA for detection of antibodies against FMDV SP and NSP

Chemiluminescence method=CLIA

- Rapid： 15-30min
- ELISA format
- Early detection: antibodies to 3ABC and 2C at 2 dpc



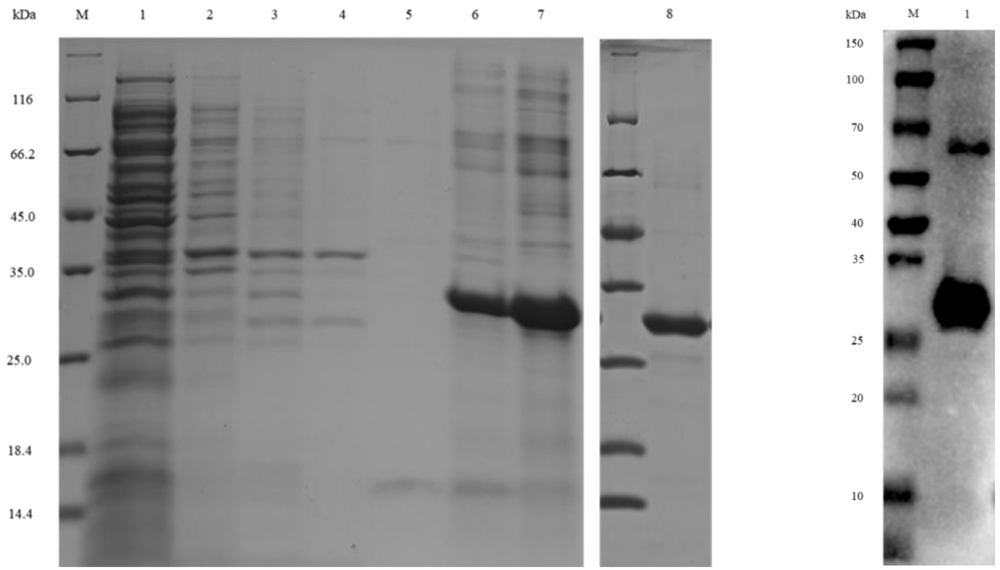
2.4 Preparation of diagnostic methods for SAT2



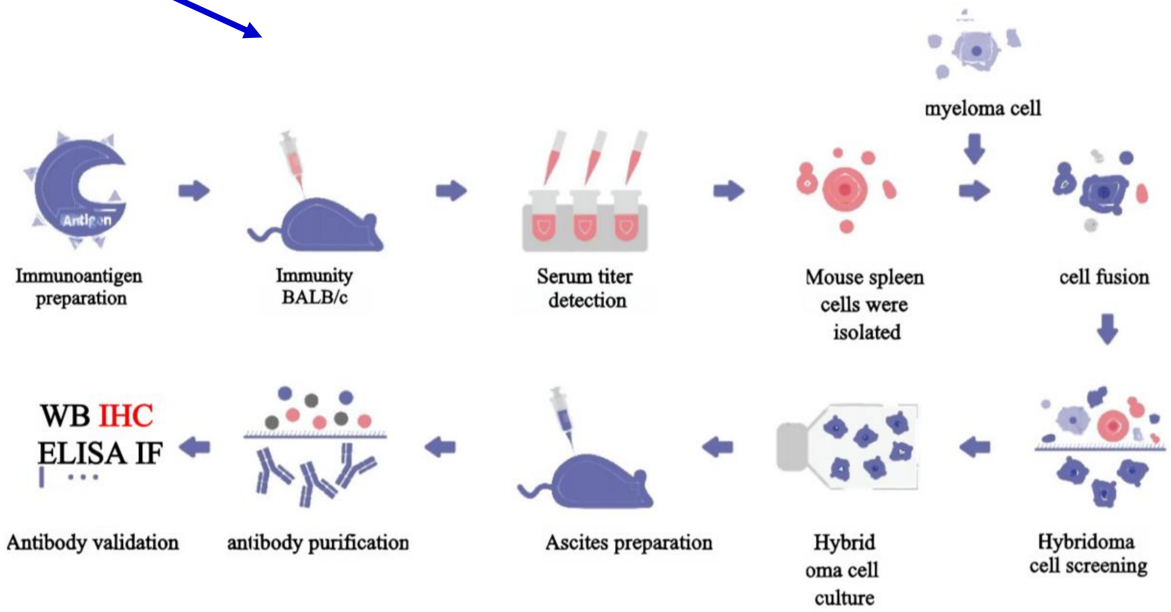
- SAT2 FMDV spread to the Middle East and West Asia;
- 2 topotypes of SAT2 FMDVs : topotype XIV and topotype VII;
- For SAT2 / XIV strain, RT-qPCR was established with the specific primers and probe recommended by WRL, and
- SAT2 antibody detection: blocking ELISA method

Preparation of diagnostic methods for SAT2

Preparation of biomaterials



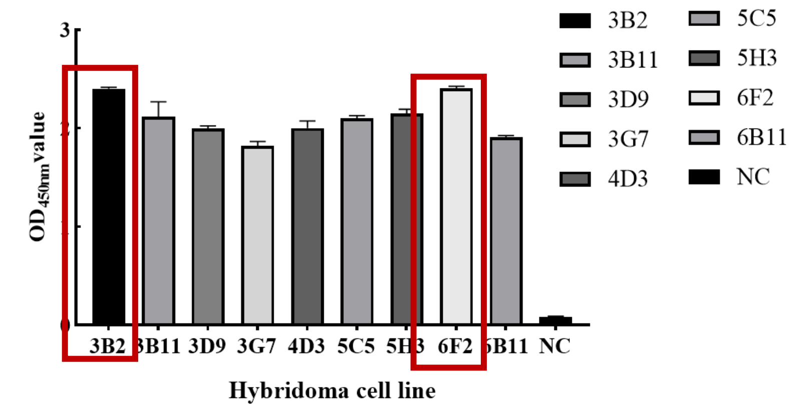
VP1 Protein expression and identification



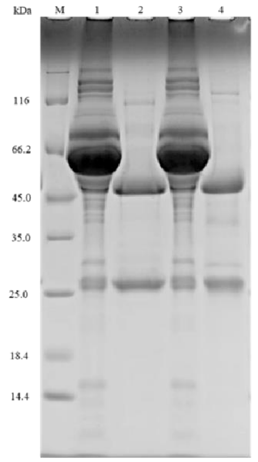
Preparation of monoclonal antibodies

Preparation of diagnostic methods for SAT2

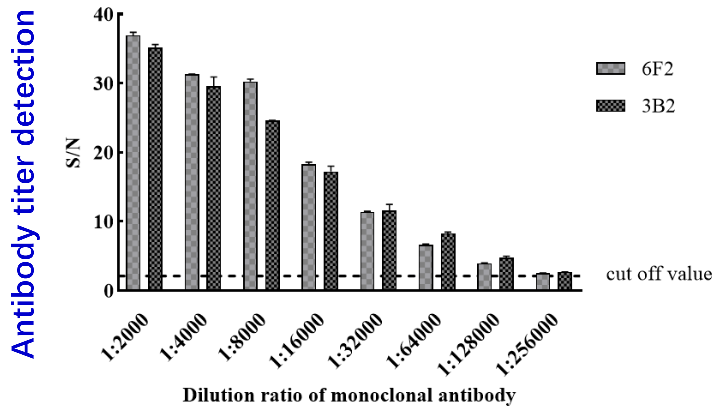
monoclonal antibodies identification

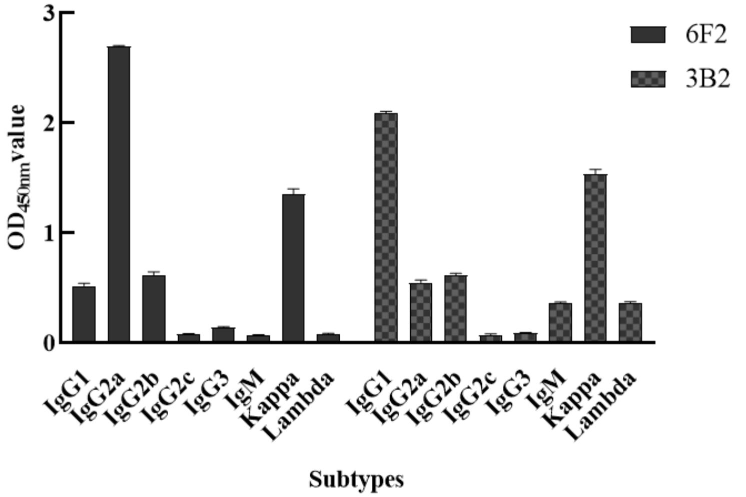


Screening of hybridoma cell lines

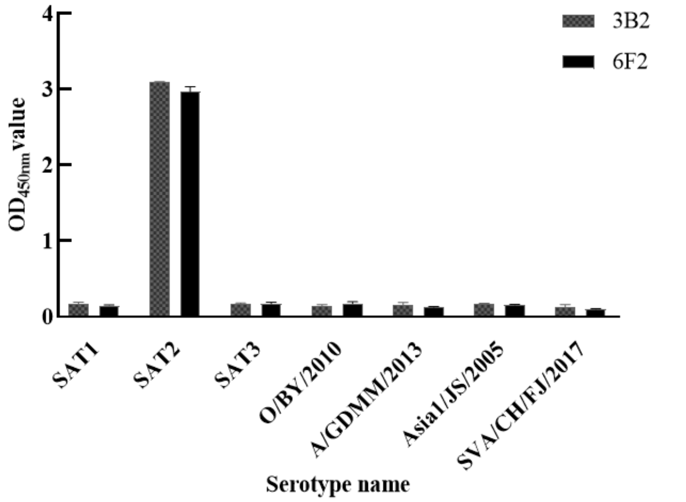


Mab purification





Subtype identification



Specificity

Preparation of diagnostic methods for SAT2

Performance test of monoclonal antibody in Pirbright

			SAT2 mab 1/10000
A	ZAM1/2015	AFRICA	0.1259
A	EGY1/1972	AFRICA	0.1156
A	KEN 3/1964	AFRICA	0.1446
A	EGY3/2016	AFRICA	0.1228
A	MAI16/2006	AFRICA	0.1214
A	ETH 12/2009	AFRICA	0.1308
A	IRN1/2016	ASIA	0.1229
A	PAK 56/2015	ASIA	0.1341
A	IRN 6/2016	ASIA	0.1372
A	CAM5/2015	ASIA	0.1366
A	SAU 15/2016	ASIA	0.1297
A	IRN1/2005	ASIA	0.1484
A	AFG 6/2007	ASIA	0.1274
A	EGY31/2014	ASIA	0.1179
A	IRN 78/2009	ASIA	0.1252
A	MYA3/2015	ASIA	0.1122
neg			0.131
Asia 1	AFG1/2001	ASIA	0.133
Asia 1	IRN 26/2016	ASIA	0.1331
Asia 1	IND 18/1980	ASIA	0.1394
Asia 1	IRN 10/2004	ASIA	0.1233
Asia 1	NKR 2/2007	ND	0.1272
neg			0.1409
neg			0.1753
C	KEN1/2004	AFRICA	0.1139
C	NEP 35/1996	ASIA	0.1145
C	PHI 3/1994	Euro-SA	0.1195
neg			0.1318

neg			0. 1073
neg			0. 1373
SAT2	MOZ3/2015		2. 8736
SAT2	ZIM 25/2015	II	2. 6949
SAT 2	BOT 3/2015	III	2. 9576
SAT2	ZAM2/2015	IV	2. 6483
SAT2	UGA9/1995	IX	2. 2746
SAT2	GHA8/1991	V	2. 9066
SAT2	OMN 3/2015	VII	2. 6467
SAT2	EGY 44/2012	VII	2. 2847
SAT2	MAU1/2014	VII	2. 1445
SAT 2	UGA2/2002	X	2. 3793
SAT 2	UGA3/1976	XII	2. 3656
SAT2	SUD 6/1977	XIII	2. 2584
SAT 3	KNP 48/1991(Buffalo)	1 (SEZ)	0. 1349
SAT3	RHO 5/1975	1(SEZ)	0. 1226
SAT 3	SAR1/2006	1(SEZ)	0. 1472
SAT 3	BOT P10/2010(buffalo)	1(WZ)	0. 1327
SAT 3	ZAM3/2015	1(WZ)	0. 136
SAT3	ZIM2/1984	1(WZ)	0. 1399
SAT3	ZIM P25/1991(UR-7 Buffalo)	II (NWZ)	0. 1319
SAT 3	ZAM P2/1996(MUL-4)	IV	0. 1375
SAT3	UGA 10/1997	V(EA-1)	0. 1469
A22cc VLP	100ng/well		0. 1578
01Mcc VLP	100ng/well		0. 1732
Asia1ccVLP	100ng/well		0. 2758

O	HKN1/2015	CATHAY	0.1249
O	TAN4/2014	EA-2	0.1526
O	EGY 36/2014	EA-3	0.1205
O	EGY 18/2016	EA-3	0.1424
O	BAN 5/2009	ME-SA	0.1259
O	KUW3/1997	ME-SA	0.1381
O	OMN 7/2001	ME-SA	0.1417
O	NEP 17/2016	ME-SA	0.1492
O	PAT 6/2015	ME-SA	0.1354
O	IRN 72/2009	ME-SA	0.1267
O	BAR 1/2014	ME-SA	0.1277
O	IRN 18/2010	ME-SA	0.1187
O	IRN 29/2013	ME-SA	0.1389
O	PAK16/2010	ME-SA	0.1269
O	KUW1/2016	ME-SA	0.1369
O	EGY 32/2009	ME-SA	0.1334
O	MAY 3/2014	SEA	0.1623
O	MYA 5/2015	SEA	0.1985
O	NIG 3/2014	WA	0.1578
neg			0.175
SAT1	KEN4/2013	I (NWZ)	0.1187
SAT1	ZIM 14/2015	I	0.1077
SAT 1	NMB 1/2015	III (WZ)	0.1116
SAT 1	UGA 7/1999	IV(EA-1)	0.1205
SAT 1	ETH 3/2007	IX	0.1272
SAT1	MOZ1/1975	ND	0.1098
SAT 1	NIG 1/1976	V	0.1091
SAT1	NIG 3/1980	VI	0.1133
SAT1	UGA 47/1971	MII(EA-2)	0.1294
neg			0.1272

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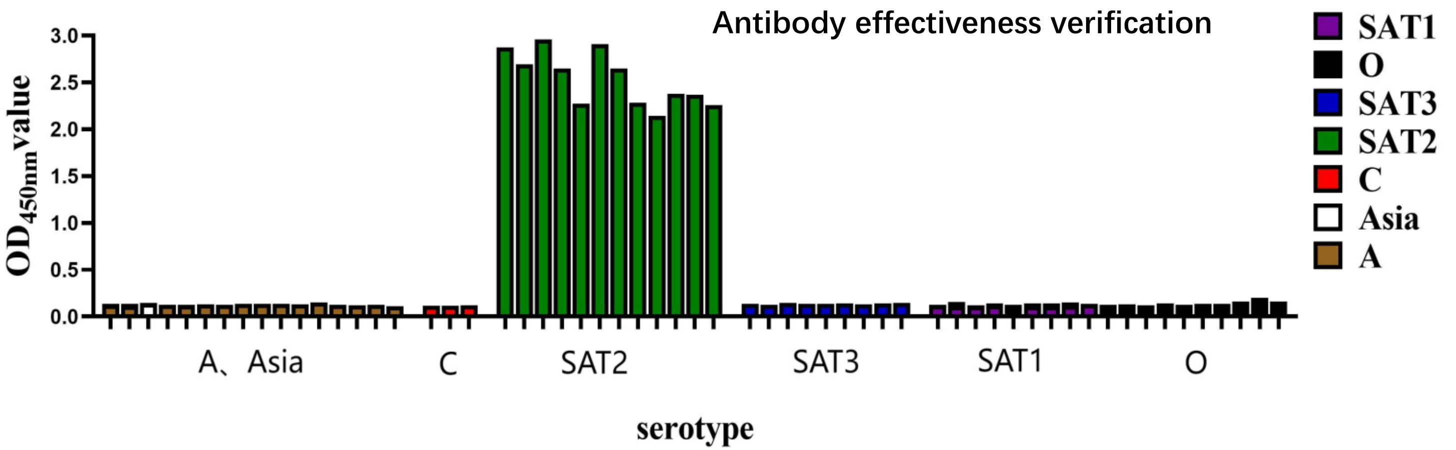
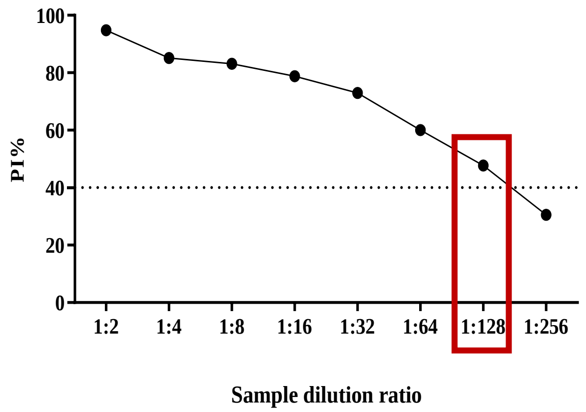


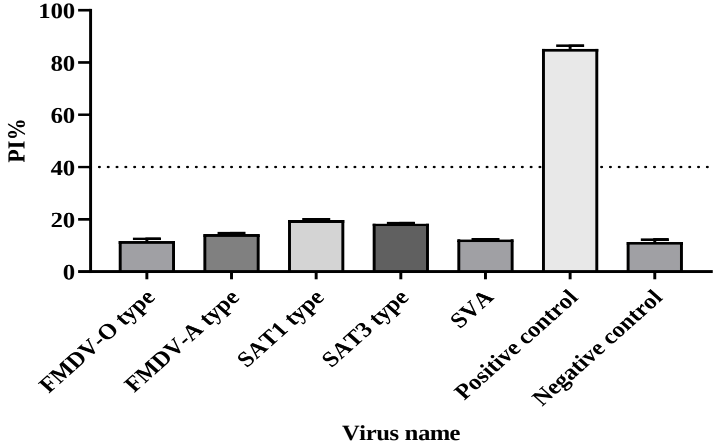
Contact： 田宏 tianhong@caas.cn

Preparation of diagnostic methods for SAT2

Sensitivity, specificity and repeatability of SAT2 antibody detection kit



Sensitivity test



Specificity test

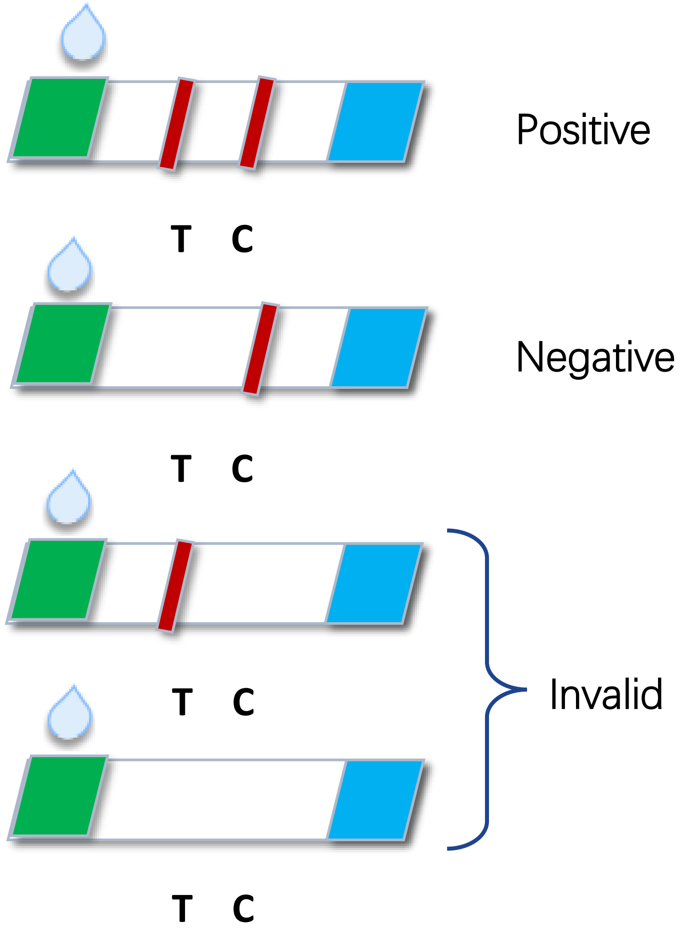
stability testing

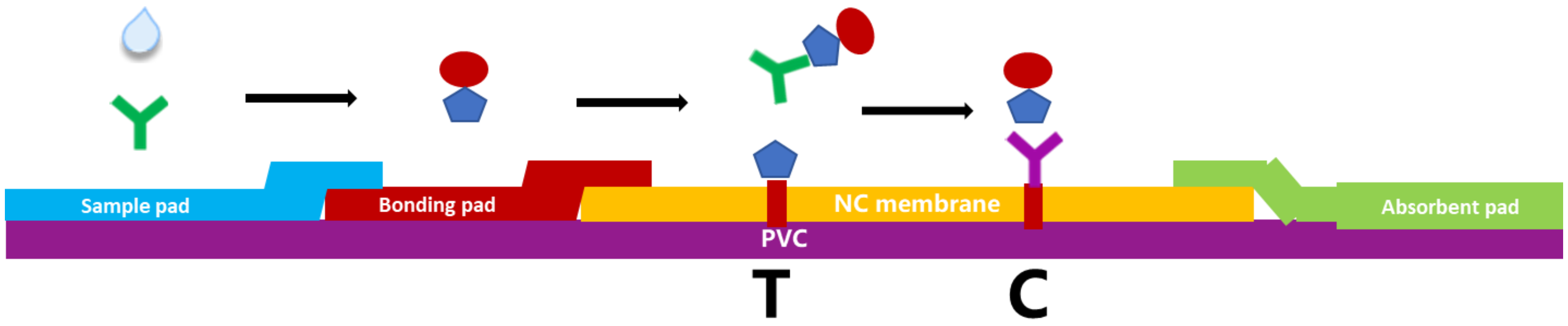
Sample number	intra-batch		Coefficient of variation/%	interlot		Coefficient of variation/%
	Mean value	SD		Mean value	SD	
1	0.334	0.003	0.750	0.289	0.007	2.355
2	0.506	0.028	5.440	0.403	0.011	2.815
3	0.555	0.013	2.254	0.551	0.008	1.491
4	0.674	0.020	2.895	0.656	0.013	2.038
5	0.740	0.009	1.216	0.593	0.027	4.583
6	0.762	0.037	4.793	0.442	0.018	4.160

Colloidal gold test strip method for SAT2

Colloidal gold test strip pattern

- VP1 protein
- Colloidal Gold
- Antibodies to be tested
- VP1 monoclonal antibody

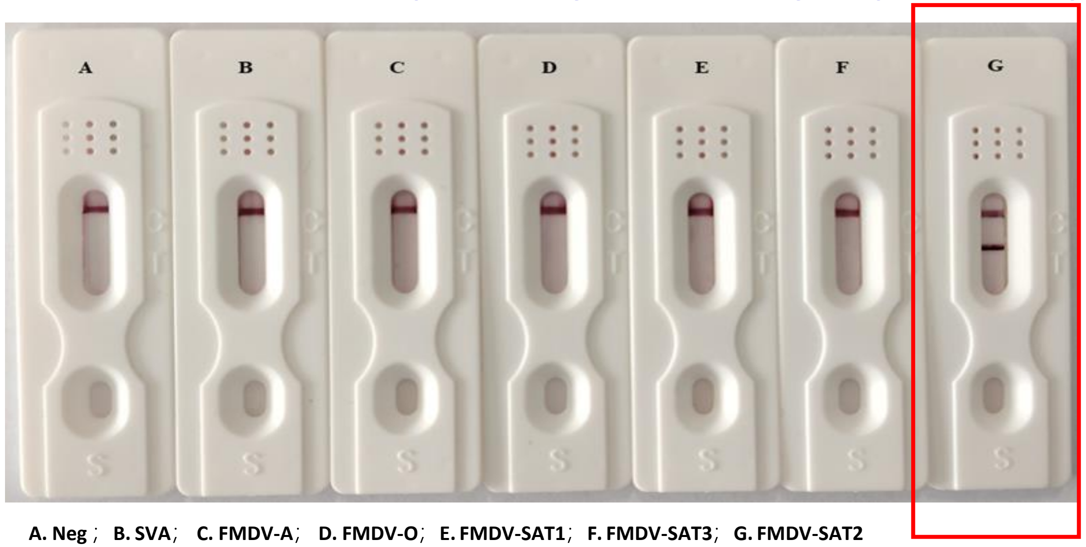




Contact: 田宏 tianhong@caas.cn

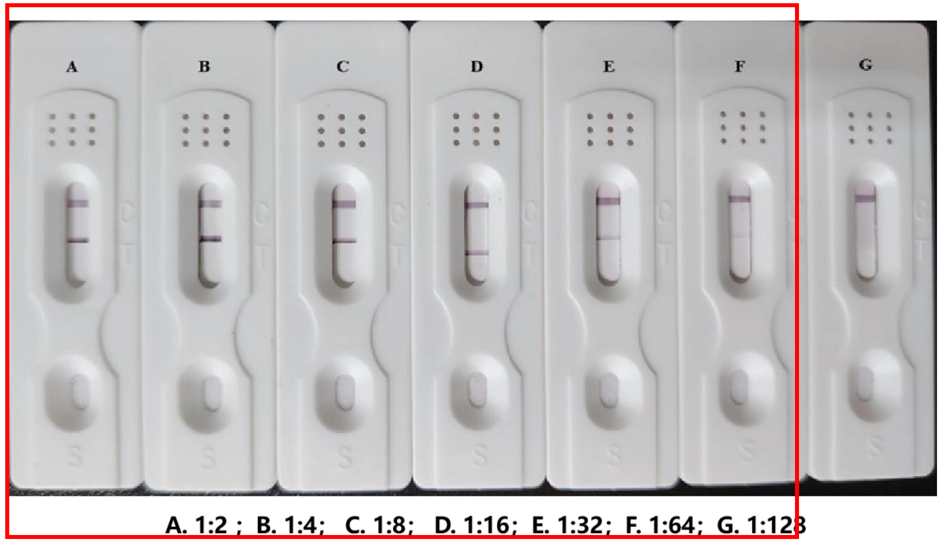
Colloidal gold test strip method for SAT2

Specificity, sensitivity, repeatability and stability of SAT2 antibody test strip



A. Neg； B. SVA； C. FMDV-A； D. FMDV-O； E. FMDV-SAT1； F. FMDV-SAT3； G. FMDV-SAT2

Specificity test

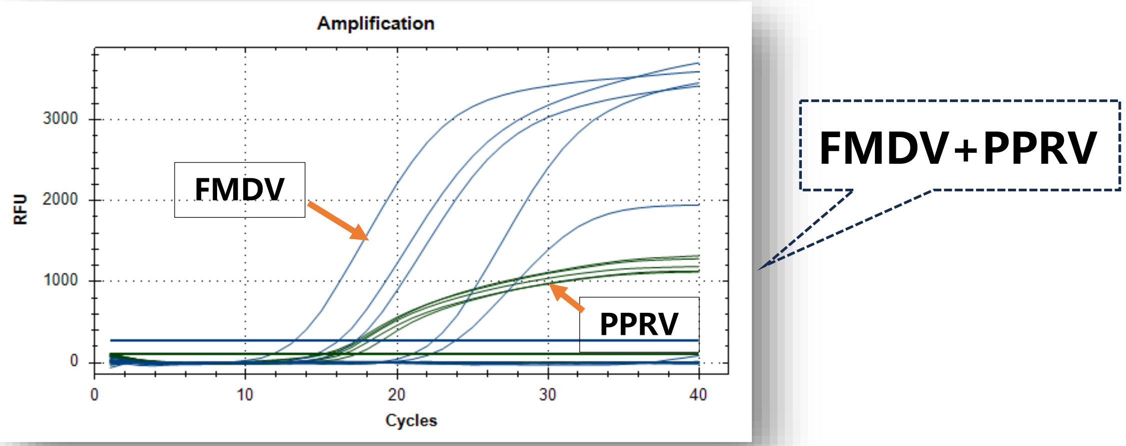


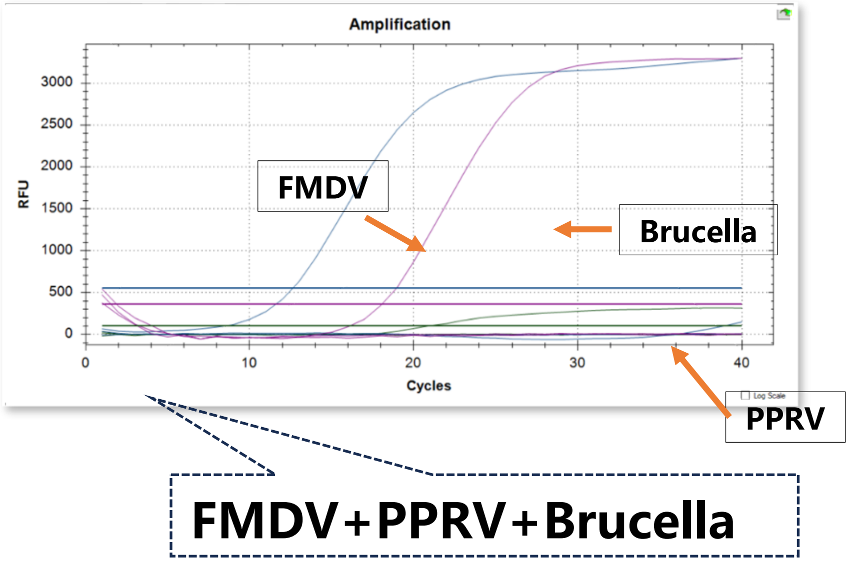
A. 1:2 ； B. 1:4； C. 1:8； D. 1:16； E. 1:32； F. 1:64； G. 1:128

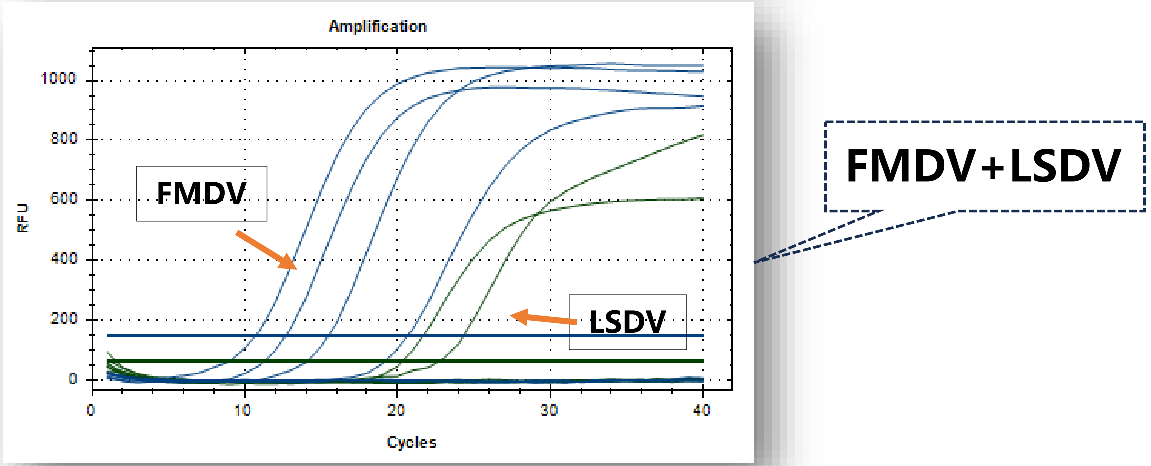
Sensitivity test

Repeatability test

2.5 other joint detection methods





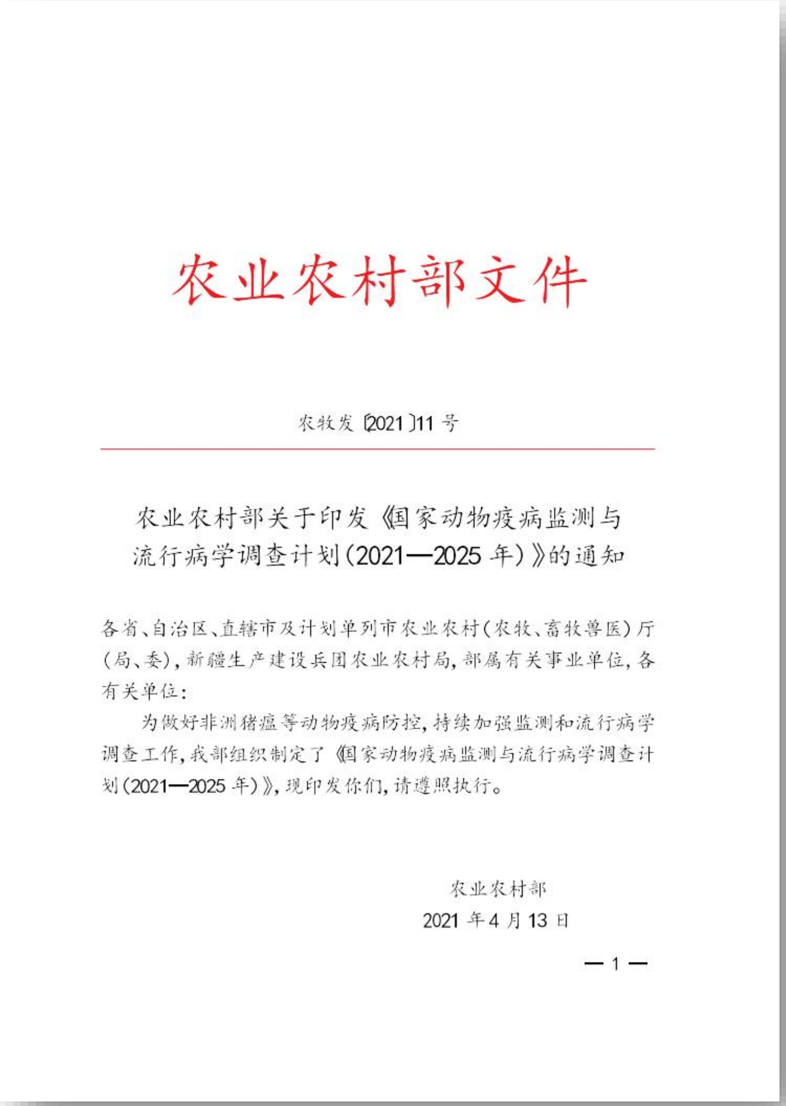


Sample	Species	Quantity	Method	Result	
				FMDV(+)	PPRV(+)
OPF	sheep	188	FMDV+PPRV	7	6

Contact：马维民 maweimin@caas.cn

- Based on the epidemiological investigation, surveillance and quarantine requirements
- co-detection methods for FMDV+ PPRV、 FMDV + LSDV and FMDV+ PPRV + Brucella
- The results show good specificity and sensitivity compared to the single method for FMDV ,PPRV, LSDV

Diagnostic technology supports FMD prevention and control



农业农村部文件

农牧发〔2021〕11 号

农业农村部关于印发《国家动物疫病监测与流行病学调查计划（2021—2025 年）》的通知

各省、自治区、直辖市及计划单列市农业农村（农牧、畜牧兽医）厅（局、委），新疆生产建设兵团农业农村局，部属有关事业单位，各有关单位：

为做好非洲猪瘟等动物疫病防控，持续加强监测和流行病学调查工作，我部组织制定了《国家动物疫病监测与流行病学调查计划（2021—2025 年）》，现印发你们，请遵照执行。

农业农村部

2021 年 4 月 13 日

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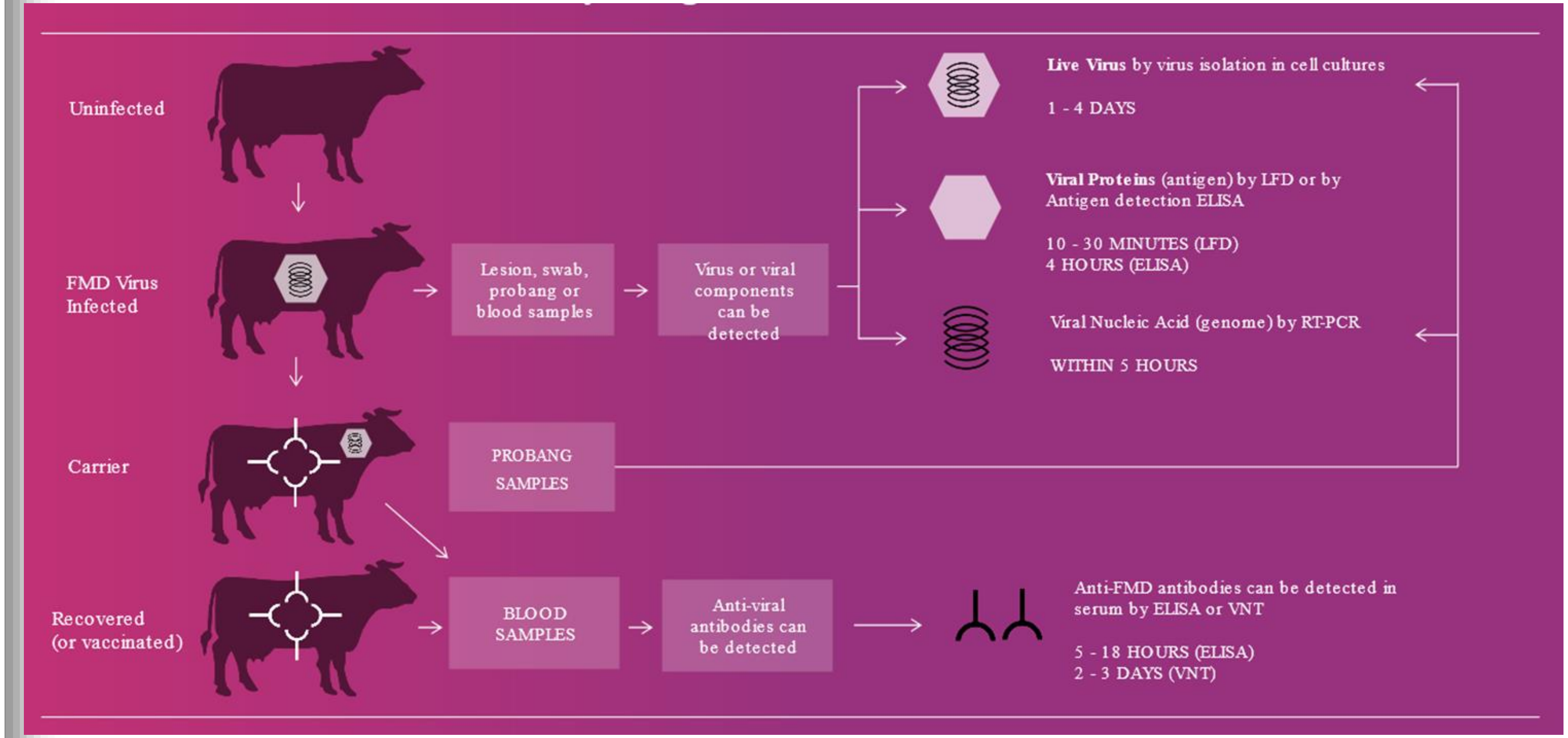

Thank You

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Lanzhou Veterinary Research Institute Chinese Academy of Agricultural Sciences

中国国家口蹄疫参考实验室
OIE/China National Foot-and-Mouth Disease Reference Laboratory