Equine-Infectious anemia virus detection technology

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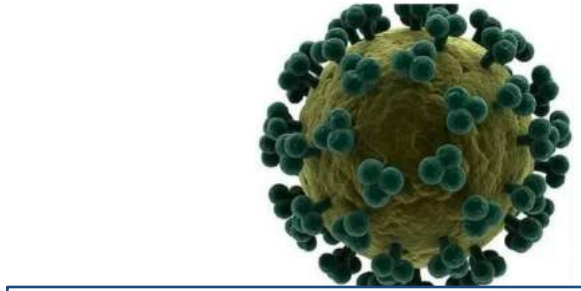
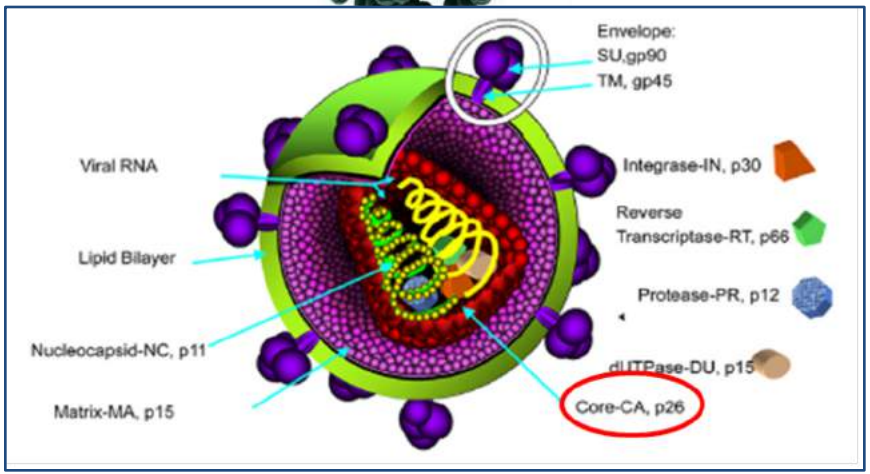
Equine infectious anemia

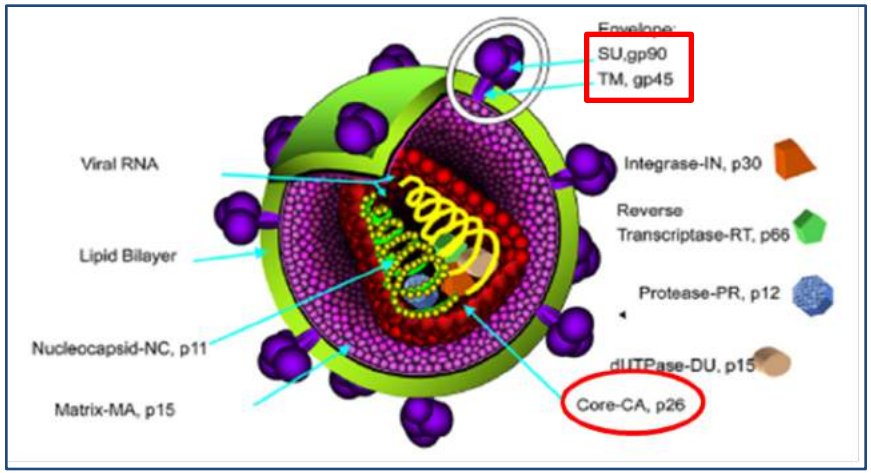
Equine infectious anemia (equine-borne anemia for short) is an infectious disease of equine animals caused by equine infectious anemia virus; equine-borne anemia virus belongs to the retroviral family lentivirus genus, and is in the same family and genus as HIV and other lentiviruses. Its characteristics are mainly intermittent fever, weight loss, progressive weakness, anemia, hemorrhage and edema; during the non-fever period, the symptoms gradually alleviate or disappear temporarily.

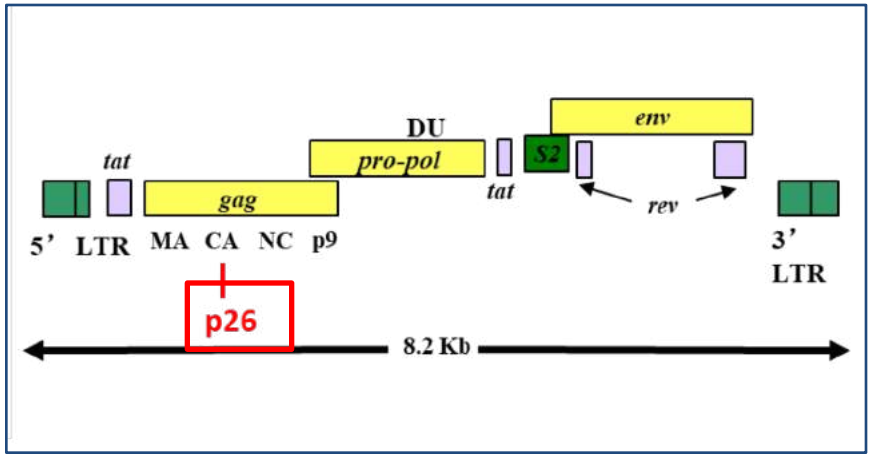


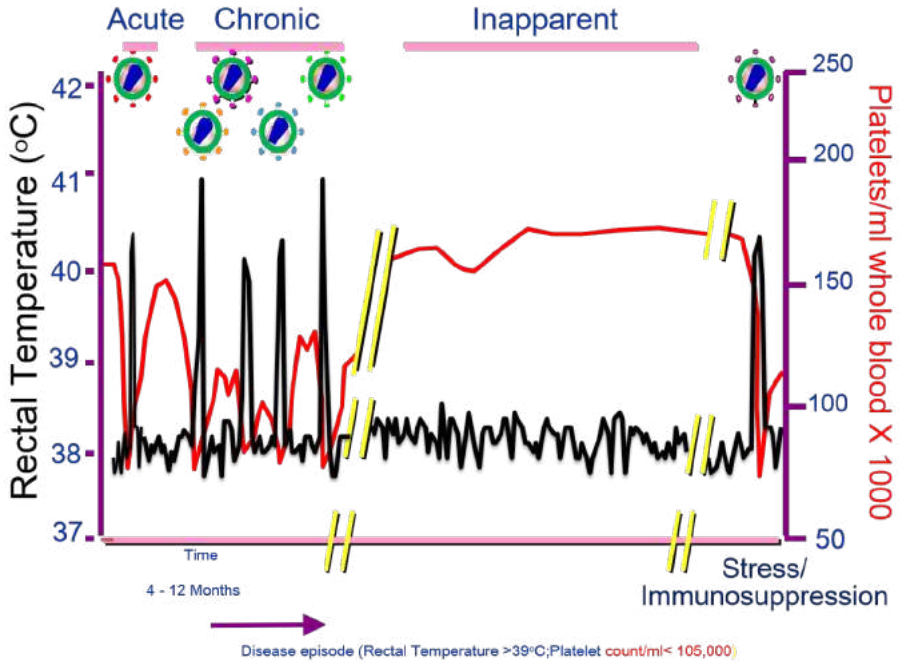


Transmission route of equine-borne anemia virus

The genome of equine-borne anemia virus and its main immunostimulants







laboratory diagnosis method of Equine Infectious Anemia

Test Method		Level of Advancement	Innovativeness	level of industrialization
clinical diagnosis		Classic method	N/A	N/A
Pathogen testing	Virus isolation and identification	Classic method	N/A	N/A
	Real-time Fluorescent PCR	internationally leading	innovative	industrialized
Serological testing	Agar diffusion test	internationally leading	innovative	industrialized
	Competitive ELISA	internationally leading	innovative	industrialized
	Western Blot Test	Classic method	N/A	N/A
	colloidal gold test	internationally	innovative	industrialized

Serological testing methods

Detection sensitivity

Enzyme-linked immunosorbent assay indirect iELISA

there can be false positive results

Western blot

confirmation method

Enzyme-linked immunosorbent assay competitive ELISA

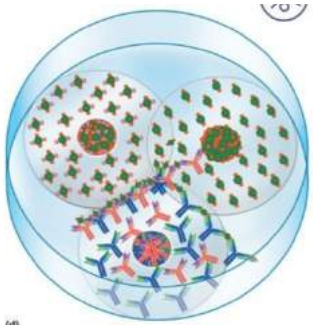
high specificity, no false positive results

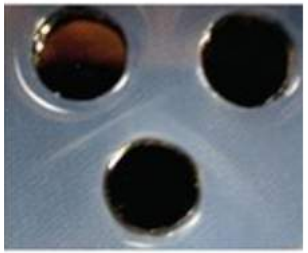
Agar immunodiffusion test

there can be false negative results

Agar immunodiffusion test

Agar diffusion test is a precipitation test of soluble antigen and corresponding antibody in semi-solid gel (agar or agarose) containing electrolyte.。



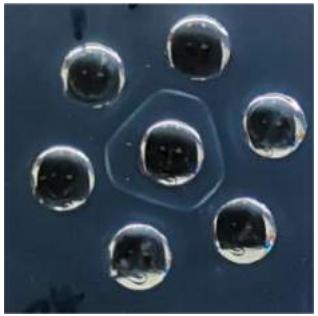


self made video by our laboratory

- AGID

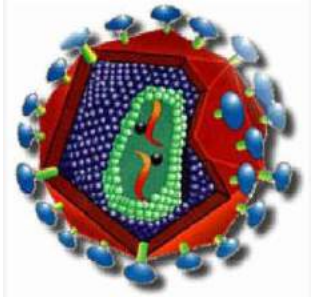
test steps：preparation → solidification → punching → picking → heating and sealing the bottom → Add serum sample after cooling

result determination：

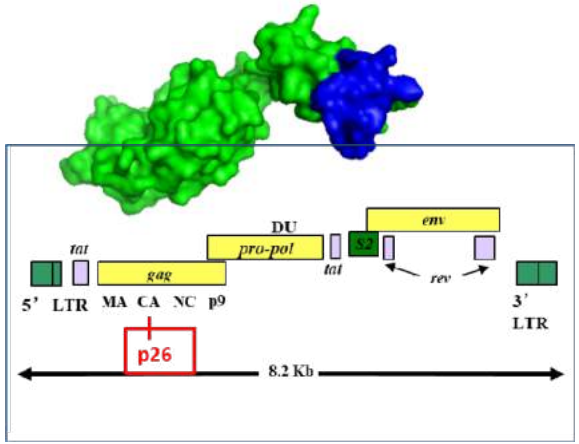


flaws：
1. Time-consuming and laborious;
2. many determining factors;
3. Poor sensitivity and low detection rate;
4. Good specificity, it's a small probability event to have false positive precipitation line

Improved agar diffusion kit for detection of equine infectious anemia antibody

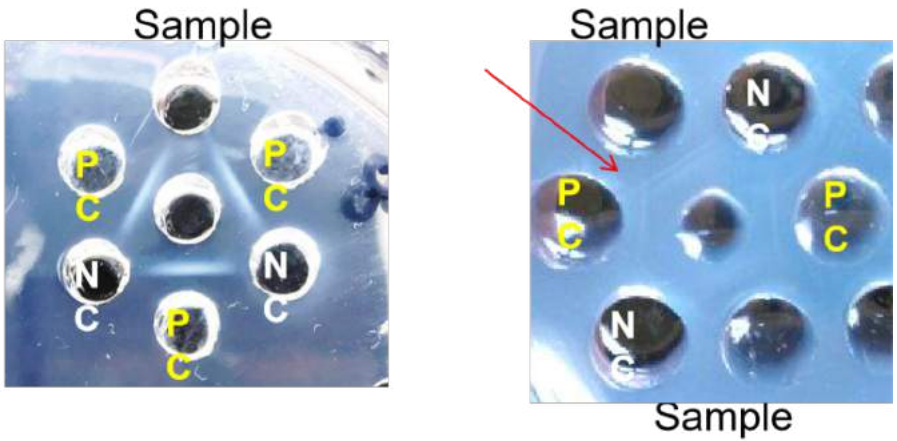


改良



HVRI upgraded AGID kit can avoid false positives

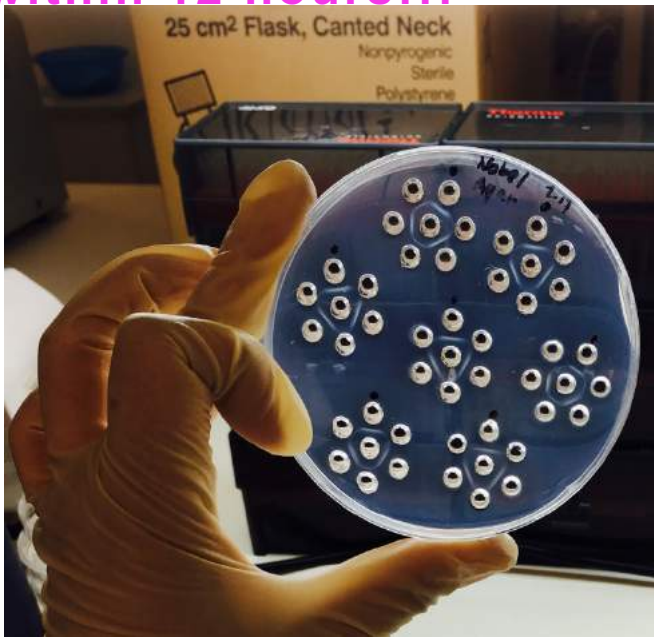
HVRI updated AGID Kit **AGID Kit**



PC：Positive control
NC：Negative control
Sample：serum sample

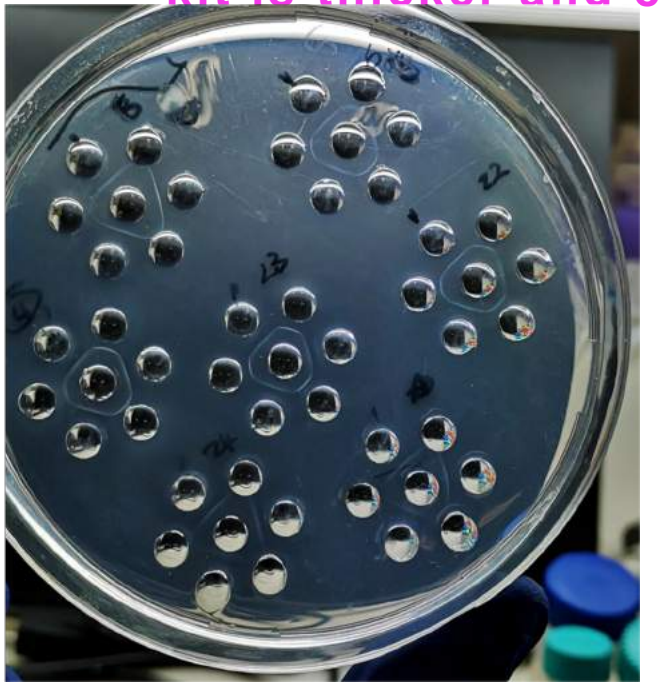
The HVRI upgraded AGID kit can have a precipitation line within 12 hours!!!

24 hours

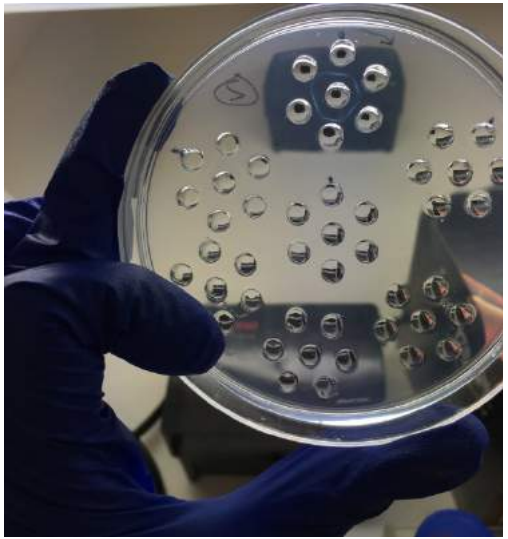


48 hours

the precipitation line of the HVRI upgraded AGID kit is thicker and easier to read!!!



HVRI updated AGID Kit



VMRD AGID Kit

Comparison of HVRI's upgraded AGID kit and American kit

Kit / Sample	AGID		
	IDEXX	VMRD	HVRI
NVSL-902	-	-	1x
NVSL-903	-	-	-
VMRD-Strong	-	-	-
VMRD-Medium	-	-	-
VMRD-Weak	-	-	-
14EIA004	8x	8x	8x
Lin	8x	-	8x
2011	8x	4x	8x
Ruo	1x	-	1x
2009	16x	8x	8x
1G11	N/A	N/A	N/A

The gold standard The disadvantages of the immunoagar diffusion test (AGID)

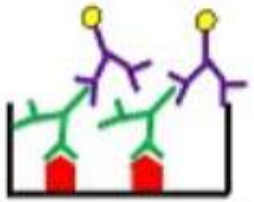
1. Poor sensitivity and high misses
2. cumbersome operation (gluing, punching, sealing, incubation, testing)
3. long operation time (24-48 hours)
4. Low throughput
5. large consumption of positive serum
6. Determination requires strong experience (determine by sight)
7. There can be false positives

Ideal serological testing method:

- fast
- High throughput
- Good specificity
- High sensitivity

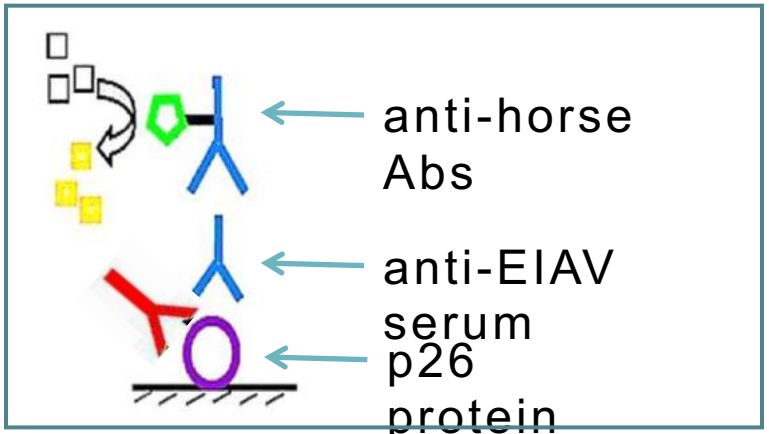
Enzyme-linked immunosorbent assay ELISA

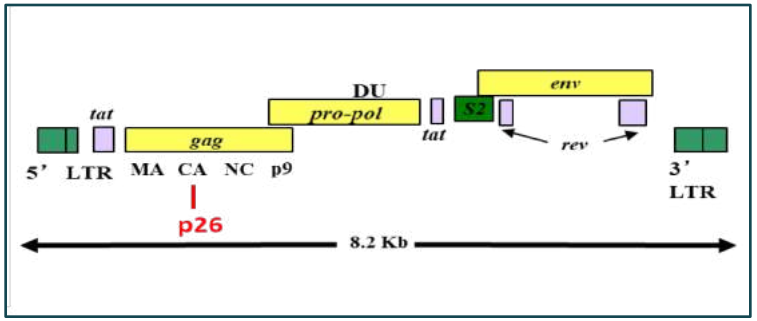
The known antigen or antibody is adsorbed on the surface of the solid phase carrier (polystyrene micro reaction plate), the enzyme-labeled antigen and antibody reaction is carried out on the solid phase surface, and the free components in the liquid phase are washed away by the washing method。



https://www.iqiyi.com/w_19ru1y0mdd.html

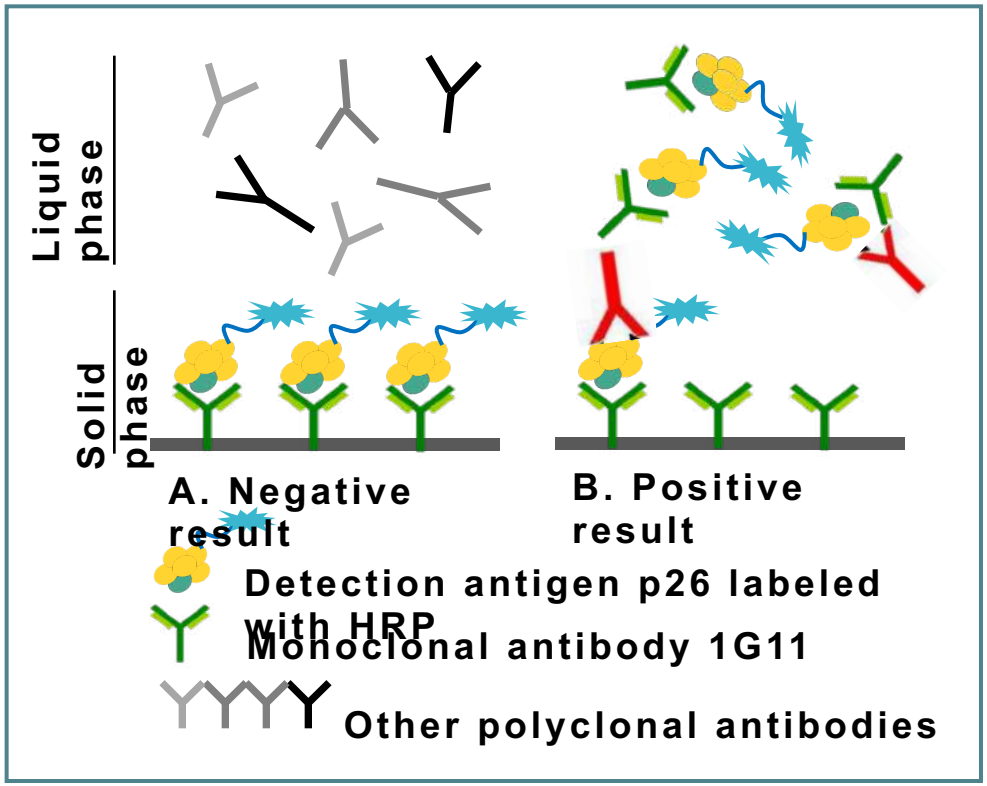
iELISA

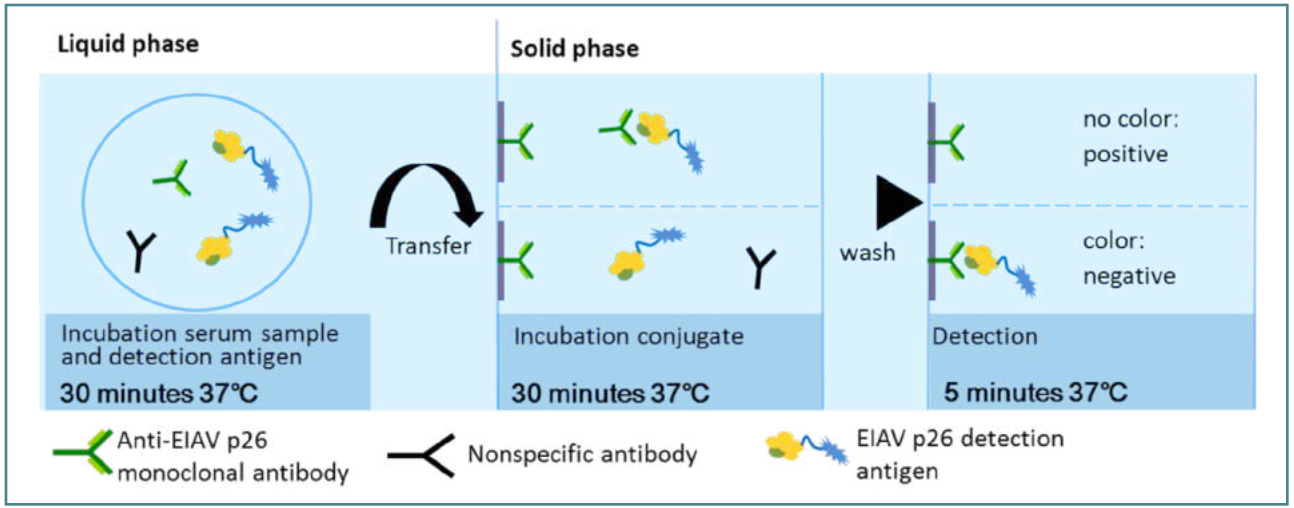




Nonspecific antibodies

cELISA





low OD value → High inhibition rate → positive

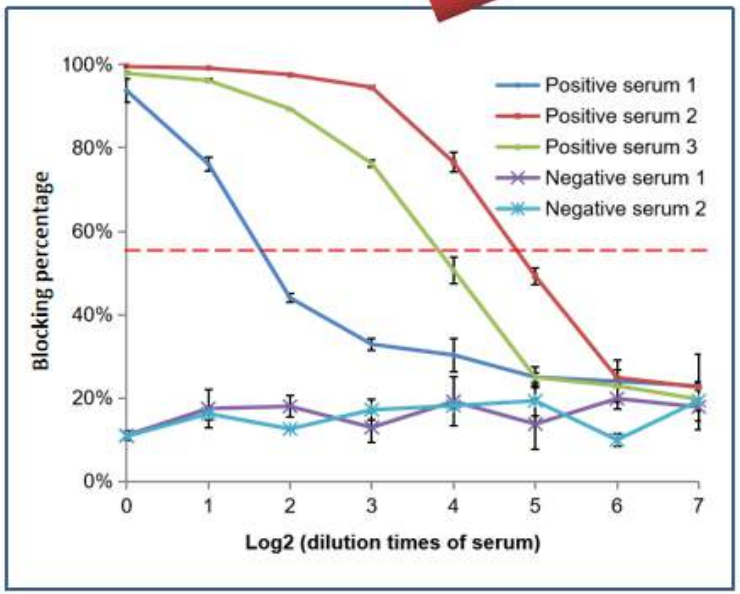
high OD value → low inhibition rate → negative

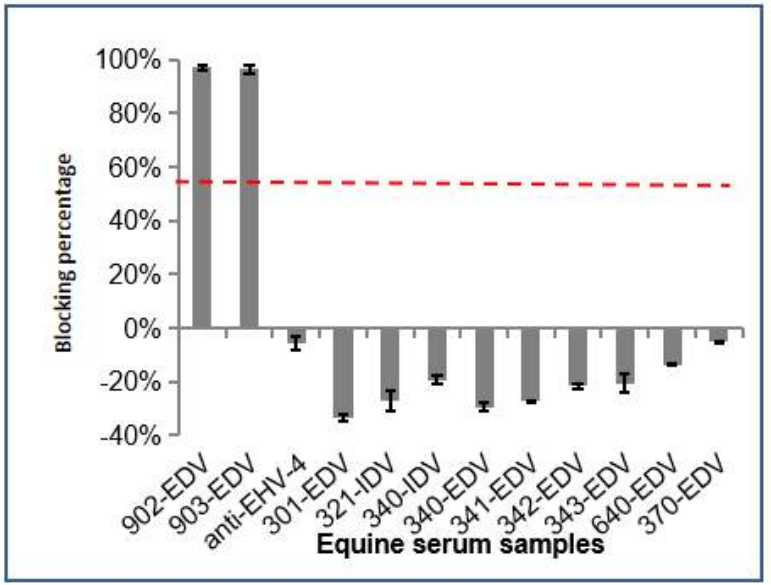
calculation formula:

$$\text{inhibition rate} = \frac{OD_{450(NC)} - OD_{450(sample)}}{OD_{450(NC)} - OD_{450(PC)}}$$

CutOff Value

Over 55% of blocking percentage is POSITIVE





The titers of antibodies against EIAV

cELISA

Sample \ kit	AGID			Competitive ELISA	
	IDEXX	VMRD	HVRI	IDEXX	HVRI
NAVL-902	-	-	1x	16x	32x
NAVL-903	-	-	-	4x	32x
VMRD-Strong	-	-	-	-	16x
VMRD-Medium	-	-	-	-	4x
VMRD-Weak	-	-	-	-	2x
14EIA004	8x	8x	8x	64x	64x
Lin	8x	-	8x	64x	128x
2011	8x	4x	8x	128x	128x
Ruo	1x	-	1x	32x	32x
2009	16x	8x	8x	128x	128x
1G11	N/A	N/A	N/A	N/A	1ug/ml

Method	Kit	VMRD Anti-EIAV		
		Strong	Medium	Weak
cELISA	HVRI	+	+	+
Blocking percentage		99.39%	88.42%	67.45%
cELISA	KIT	-	-	-
iELISA	VMRD	+	+	+
AGID	KIT	-	-	-
AGID	VMRD	-	-	-
Western blot	1000 dilution of serum			

"x" stands for the most dilution fold for the detection.

Use cELISA equine infectious anemia antibody detection kit to detect horse serum

Time point	Two months post immunication				Three months post immunication				One months post infection				Three months post infection			
Horse No.	Sample No.	IDEXX-AGID	HVRI-cELISA	HVRI-cELISA %	Sample No	IDEXX-AGID	HVRI-cELISA	HVRI-cELISA %	Sample No.	IDEXX-AGID	HVRI-cELISA	HVRI-cELISA %	Sample No	IDEXX-AGID	HVRI-cELISA	HVRI-cELISA %
#2	31	-	+	92.06	46	-	-	45.79	106	-	+	61.42	150	-	+	79.24
#3	32	-	+	74.79	47	-	-	51.34	107	-	-	21.93	151	+++	+	99.94
#5	30	-	+	96.34	48	++	+	100.00	108	-	+	93.10	152	-	+	97.44
#8	34	-	+	85.29	49	-	+	67.77	109	-	+	59.83	153	-	-	37.73
#14	35	-	+	93.71	50	-	+	95.24	110	-	+	70.63	154	-	+	68.32
#4	36	-	-	14.59	51	-	-	16.30	111	-	-	14.29	155	++++	+	100.12
#6	37	-	-	-1.77	52	-	-	44.08	112	-	-	11.36	156	-	+	55.13
#13	38	-	+	81.39	53	-	+	72.41	113	-	-	7.39				
#16	39	-	-	42.25	54	-	-	11.54	114	-	-	36.94				
#1	40	-	+	76.56	55	-	+	58.79	115	-	-	29.24	157	-	+	99.08
#21	41	-	-	17.09	56	-	-	14.77	116	-	-	10.56	158	-	+	75.34
#9	42	-	+	58.06	57	-	-	50.18	117	-	-	21.06				
#7	43	-	+	68.55	58	-	-	41.24					159	++	+	98.35
#10	44	-	+	88.69	59	-	-	48.69	119	-	-	13.00	160	+	+	100.12
#11	45	-	+	58.79	60	-	-	36.20	120	-	-	18.80	161	-	+	98.29
Control		+++	+	100.06												

Comparison tests carried out among 4 international laboratories, China’ s horse-borne anemia competitive ELISA method is the best

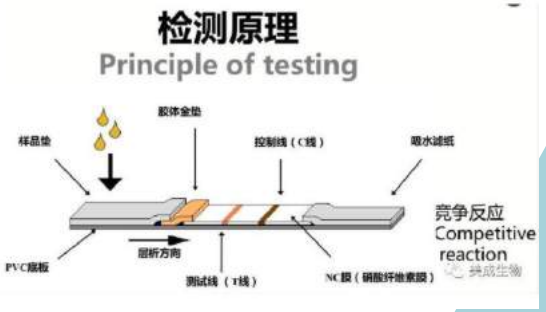
评测单位	第三方检测单位	用于比对的试剂盒	评测样品	评测结果
INGENASA	西班牙雅雷萨(INGENASA)公司	cELISA方法	ELISA强阳性血清1份，ELISA弱阳性血清3份，阴性血清4份	诊断敏感性100%，诊断特异性80%
		INGEZIM ANEMIA DR（间接ELISA）		诊断敏感性100%，诊断特异性100%
IEIDC	Instituto de Virología阿根廷病毒研究所	agar gel immunodiffusion (IDGA)	183份临床样品	诊断敏感性100%，诊断特异性84%(8+)
		美国IDEXX cELISA试剂盒	183份临床样品	诊断敏感性99.3%(1-)，诊断特异性91.1%(4+)
	香港特别行政区政府渔农自然护理署	cELISA方法	40份能力验证样品（美国）	诊断敏感性100%，诊断特异性100%
		美国IDEXX cELISA试剂盒		诊断敏感性100%，诊断特异性100%
		美国AGID试剂盒		诊断敏感性100%，诊断特异性100%
ARC • LNR	南非农业研究委员会	cELISA方法	1份阴性血清，5份阳性血清，163份临床阴性血清样品	诊断敏感性100%，诊断特异性100%
		美国IDEXX cELISA试剂盒		诊断敏感性83.3%，诊断特异性100%
		Erdikit-ELISA		诊断敏感性83.3%，诊断特异性77.3%
		美国IDEXX AGID		诊断敏感性83.3%，诊断特异性100%

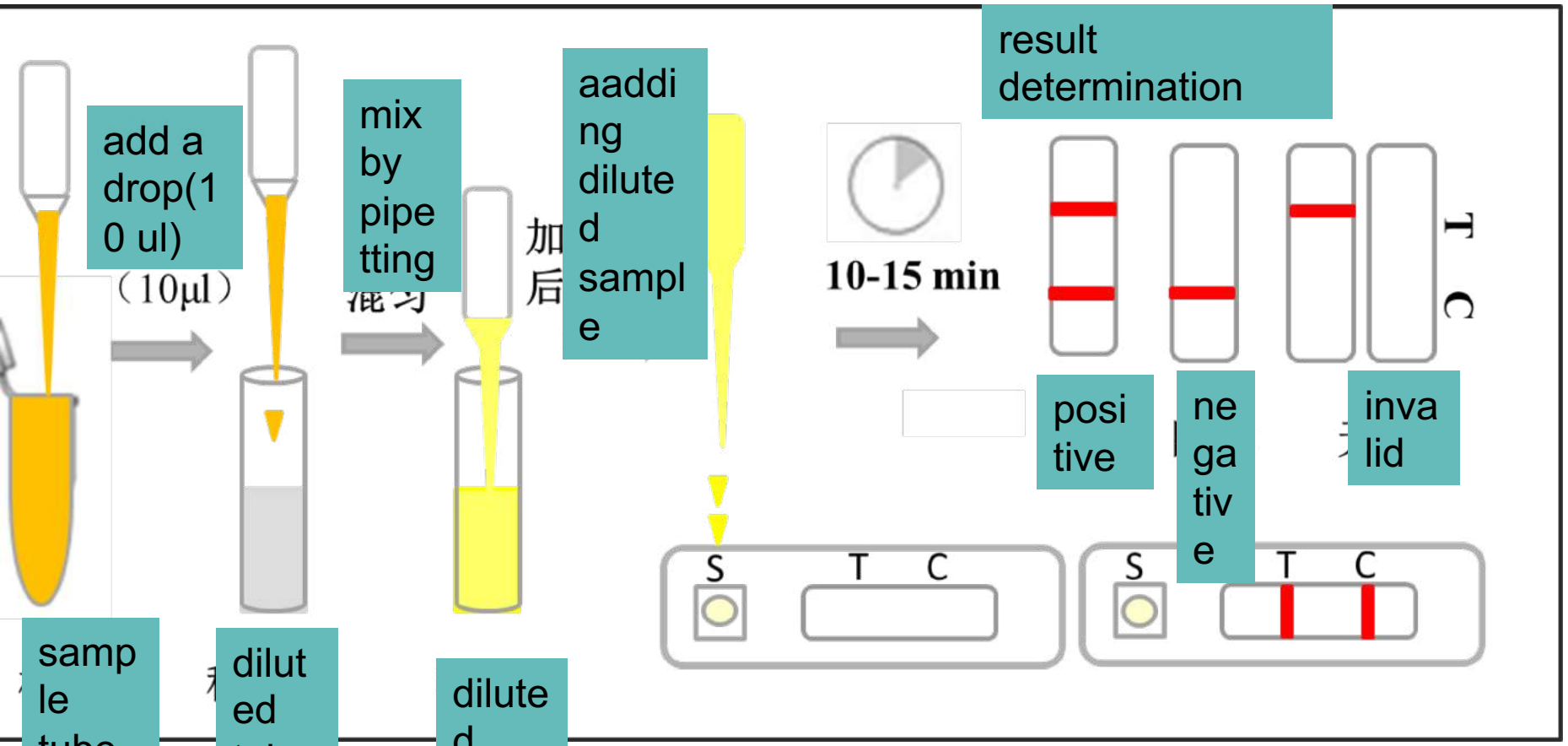
cELISA plan

high specificity	high sensitivity	rapid	high throughput
No false poitive	8 times more sensitive than AGID	Finish within 1.5 hours	500 test for each time by one person

Colloidal gold detection

Colloidal gold is a commonly used labeling technology, a new type of immunolabeling technology that uses colloidal gold as a tracer to apply to antigen and antibody。




add a drop(10 ul)
（10μl）
mix by pipetting
aaddi ng dilute d sampl e
result determination
10-15 min
posi tive
ne ga tiv e
inva lid
S
T
C
sample tube
dilut ed tube
dilute d tube

Antibody Detection Method using Colloidal Gold Test Strip for Equine Infectious Anemia

table1. good specificity

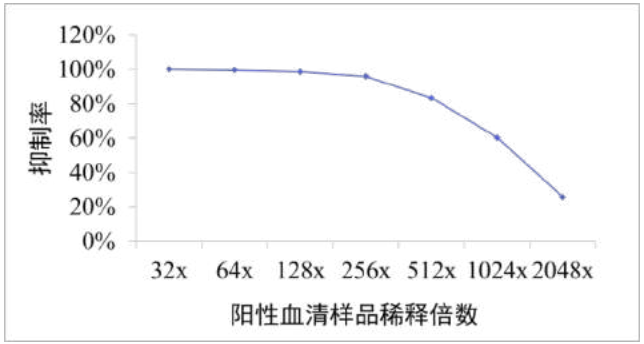


table2. Equine infectious anemia cELISA antibody detection kit detects standard positive serum

table3. high sensitivity

table4. Good repeatability

The colloidal gold test strip is two times more sensitive than the cELISA method, and can be used for rapid on-site detection and preliminary screening of a large number of clinical samples.

Pathogen detection method

OIE马传染性贫血
参考实验室

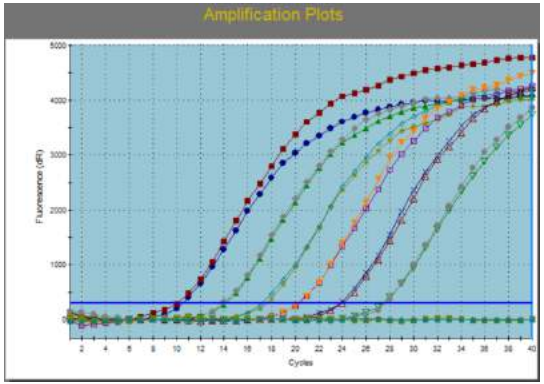
OIE Referece Laboratory for
Equine Infectious Anemia

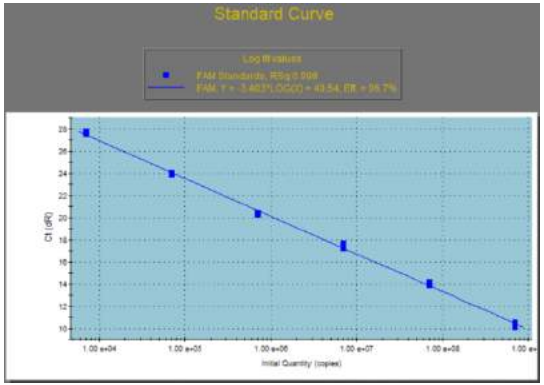
国家马传染性贫血
参考实验室

National Equine Infectious
Anemia Reference Laboratory

- Real-time fluorescence quantitative PCR

——2020 update





	OIE set	New set
Bau Gard co	-	+
cornwall	-	+
DE Italy	-	+
Devon	-	+
Ecl Gard co	-	+
F2	+	+
Ita-1	-	+
Miyazaki2011-A	-	+
Newmarket	-	+
POCONE-BRA1	-	+
SA-Italy	-	+
UK	+	+
V26	+	+
Liaoning	-	+

These methods will be incorporated into China's standardization system

Test method recommended by OIE manual		original standard	new standard	Conformity with OIE standard
Agar diffusion test		✓	✓ ✓	Conformed and superior
ELISA method	competitive ELISA	✓		Conformed and superior
	non-competitive ELISA		unr ✓ ed	Reason: false positive in indirect method
Western blot	Western blot		✓	conformed
Nucleic acid amplification testing	common PCR		unrevised	Reason: Sensitivity is lower than fluorescence method

Thank you for your attention!