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URUMQI CUSTOMS DISTRICT P.R.CHINA

Laboratory Diagnosis Technology of Equine Pyriformis

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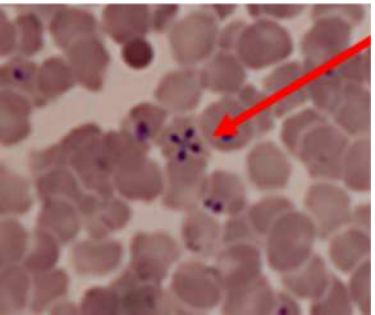
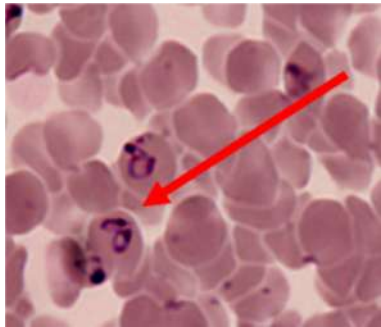
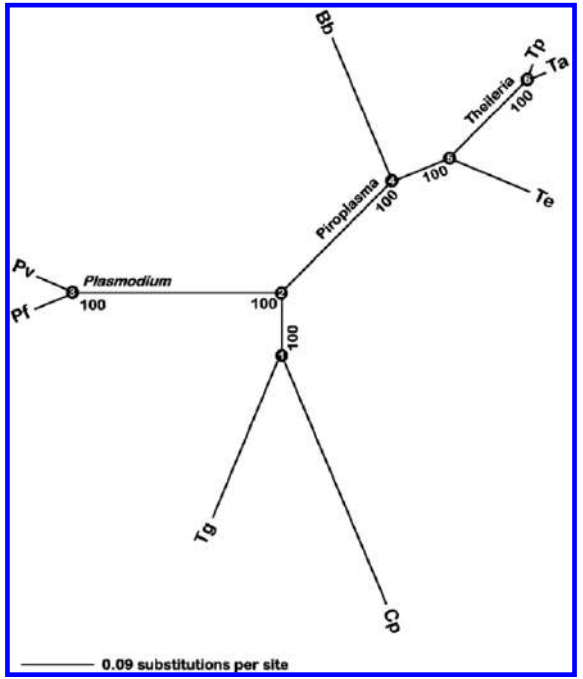




- Equine pyriformiasis: It is a kind of protozoa disease carried by ticks that parasite the red blood cells of equine animals such as horses, donkeys, mules, and zebras by two protozoa of the genus Theileria and Babesia of the genus Babesia. The spread of protozoal diseases mainly occur in tropical and subtropical regions.
- China classifies it as a second class of animal disease, and OIE lists it as a notifiable animal disease, which is one of the two types of parasitic diseases that must be inspected.
- According to the clinical symptoms of infected horses, it can be divided into acute, subacute and chronic. Although horse theileriasis and Babesiosis are different, their general clinical symptoms are similar, including fever, anemia, loss of appetite, edema, and jaundice. , Hepatomegaly, splenomegaly, and in some cases can even cause death.



Equine pyriformia and Plasmodium belong to the same phylum Apicomplex, and equine pyriformiasis is called "equine malaria"

Equine infectious diseases notified regularly by the World Organization for Animal Health (OIE)

世界动物卫生组织通报克罗地亚和韩国疫情

2017-11-23 08:17:00 来源：食品伙伴网

食品伙伴网讯 据世界动物卫生组织（OIE）消息，近日世界动物卫生组织通报克罗地亚和韩国分别发生马巴贝斯虫病（Equine piroplasmosis）、高致病性禽流感疫情。

克罗地亚发生马巴贝斯虫病

克罗地亚农业部向OIE报告称，2017年11月2日，萨格勒布县（Zagrebacka）发生马巴贝斯虫病疫情。实验室检测发现，有38匹马疑受感染，其中1匹马发病，1匹马死亡，宰杀销毁1匹。本次疫情感染来源尚不清楚。克罗地亚已采取接种、处理病马等措施。

Infectious diseases that must be checked for sports events related to horse racing, such as the Olympics and Asian Games

农业部办公厅关于印发《奥运期间动物卫生及动物产品安全监管工作方案》的通知

2008-04-22 17:39:23 来源：SRC-28

（四）奥运参赛马匹监管

1. 完善应急措施

工作内容：北京、上海、广东等省市要组织制定奥运期间参赛马匹突发疫情应急预案。北京兽医主管部门要协调有关部门确立应急隔离场、备选训练场地和备选马匹。

工作分工：北京、上海、广东三省市兽医主管部门牵头，三省市动物卫生监督所配合。

完成时限：2008年5月 - 9月

2. 限制赛场及周边地区马属动物移动

工作内容：北京、天津、河北等省（市）要报请地方政府采取有效措施，禁止辖区内马属动物跨县区移动，必要时在主要交通要道设置公路动物卫生监督检查站，加强对途径马属动物的监管，防止马流感疫情发生和跨区域传播。

工作分工：北京、天津、河北三省市兽医主管部门牵头，三省市动物卫生监督所配合。

完成时限：2008年5月 - 9月

3. 强化参赛马匹检疫监管工作

工作内容：北京、上海、广东三省市要积极协调、相互配合，结合赛事安排和赛马训练需要，针对马鼻疽、马传贫、马巴贝斯焦虫、马流感等重点疫病，切实做好赴京参赛马匹的检疫工作。参赛马匹进京后，要在北京市动物卫生监督所监督下进行隔离观察。比赛期间要对饲养、比赛场所实施严格消毒，对参赛马匹进行全程监管。

工作分工：由北京、上海、广东三省市动物卫生监督所负责。

完成时限：2008年5月 - 9月

infectious diseases that must be inspected in the construction of epidemic-free areas for equine animals

Conghua Epidemic-Free Zone in Guangzhou-2010 Guangzhou Asian Games

被动监测疫病种类

动物种类/传播媒介	监测病种
马属动物	非洲马瘟、尼帕病、亨德拉病、西尼罗河热、马传染性贫血、马鼻疽、马脑脊髓炎、马梨形虫病、马病毒性动脉炎、马媾疫、伊氏锥虫病、水泡性口炎、马流行性感冒、日本脑炎等 14 种疫病
生猪、野猪	日本脑炎、伊氏锥虫病、水泡性口炎、尼帕病毒病
牛、羊	伊氏锥虫病、水泡性口炎
蝙蝠	尼帕病毒病、亨德拉病毒病
鸟	西尼罗河热、日本脑炎
蚊	西尼罗河热、日本脑炎、水泡性口炎

Hangzhou Tonglu Epidemic-Free Zone-
2022 Hangzhou Asian Games

规定马属动物疫病 包括马传染性贫血、马鼻疽、日本脑炎、马梨形虫病、马病毒性动脉炎、马媾疫、伊氏锥虫病（苏拉病）、马流行性感冒等8种动物疫病。

Equine pyriformiasis is one of the infectious diseases requring mandatory inspection in the import and export inspection of horses

Journal of Equine Veterinary Science 92 (2020) 103152

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

The First Report of Serological Detection of *Babesia caballi* by cELISA in a Horse During Serological Survey of Piroplasmosis in Imported Horses at Shanghai Port, China

Yan Wang [a,*], Lei-Ping Zhang [a], Jian Li [a], Dan Dan Li [b], Qiang Zhang [a], Chunyang Li [a]

[a] Shanghai Customs, Shanghai, China
[b] Haikou Customs, Haikou, China

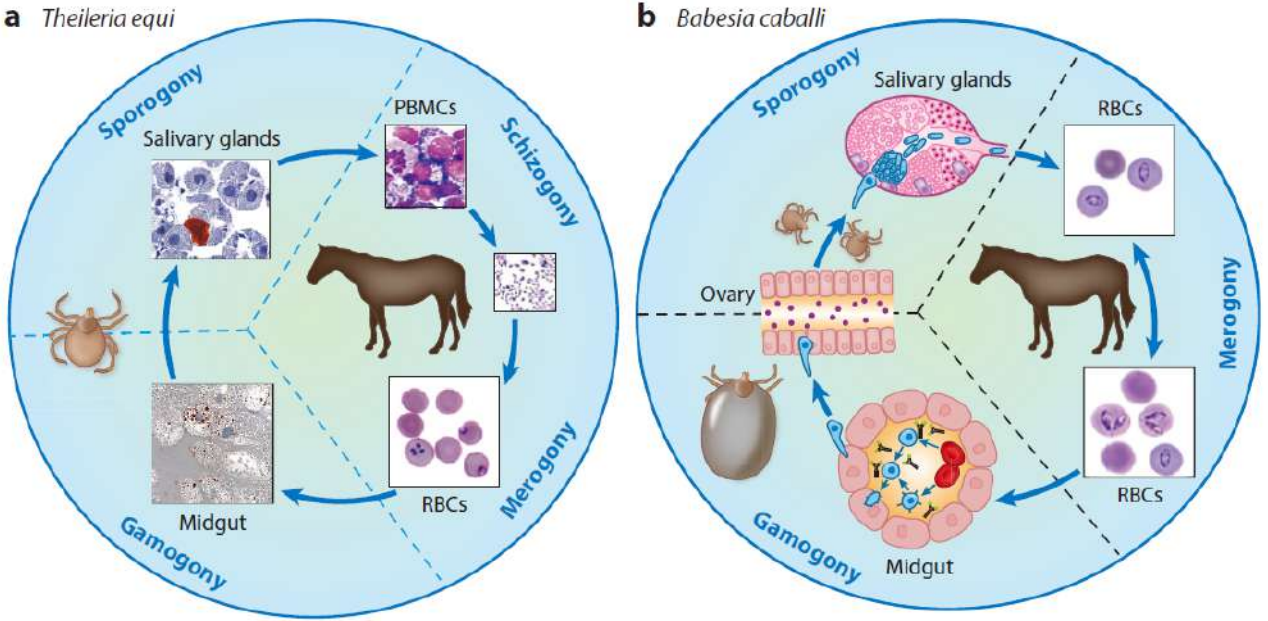
Aquis Farm雅士牧场赛马新闻：受马梨形虫病影响新西兰暂停向澳大利亚出口马匹

2020-05-21 21:04

5月21日，英国《赛马邮报》(Racing Post)旗下的ANZ Bloodstock发文称昨日（5月20日）新西兰初级产业部(Ministry for Primary Industries)通知出口商：暂停向澳大利亚出口(马匹)，立即生效(shipments to Australia had been suspended with immediate effect)。目前澳大利亚与新西兰签署的进口要求是三年，这意味着2023年以前澳新之间的马匹运输将终止。

Life history

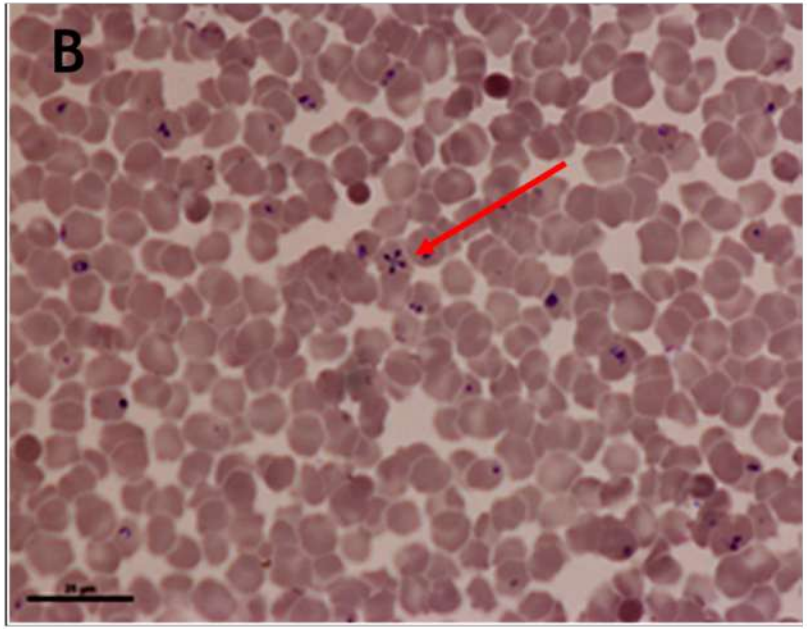
Carried for life, after being cured, it will become a recessive transmission host, and it will become ill under stress conditions.

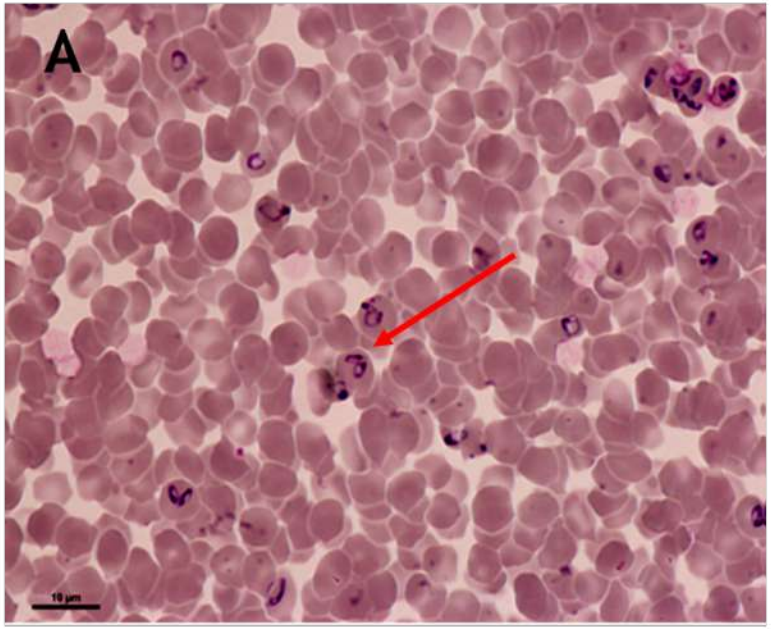


It will be cleared by the host's immune system within 3-4 years, but there are also cases of lifelong carriers.

Scoles, G. A.Ueti, M. W. et al. Annu Rev Entomol 2015

Morphological characteristics

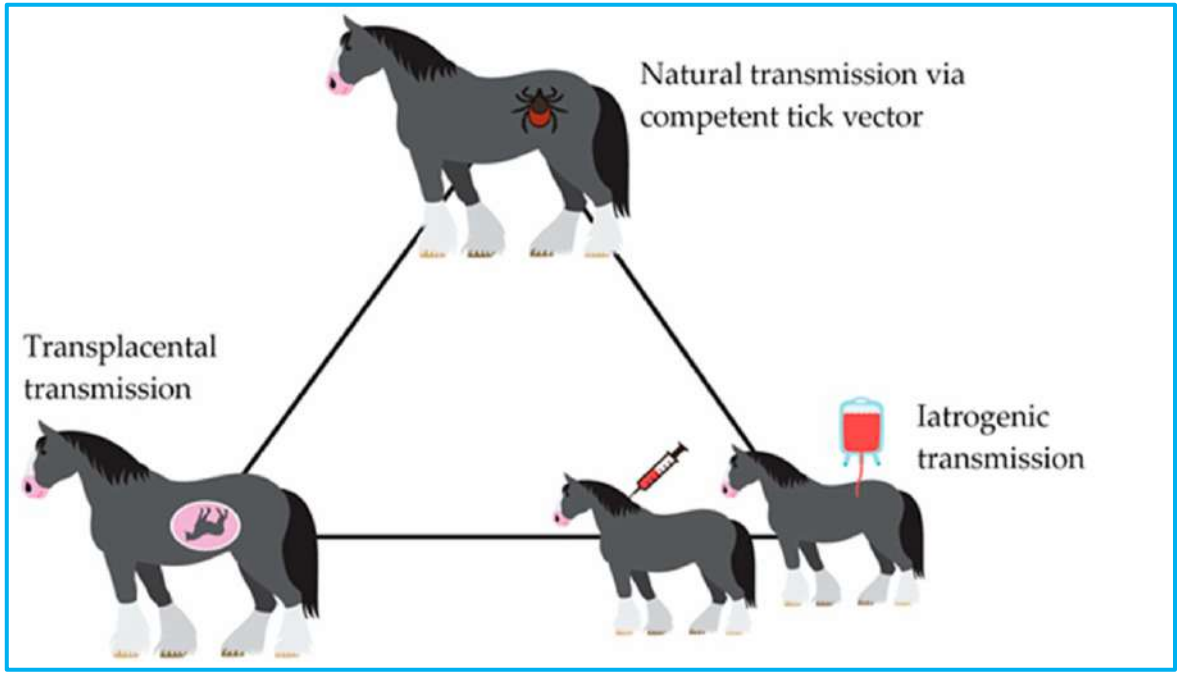




The size of the Theileria equi worm is generally 2-3um, and the typical shape of the worm is 4 pear-seed shaped body forms connected by the tips to form a cross.

Compared with Theileria equi , the body of Babesia crossbow is larger, and from the morphological observation, the most obvious feature of Babesia crossbow is that the length of the body is generally greater than the radius of

transmission route

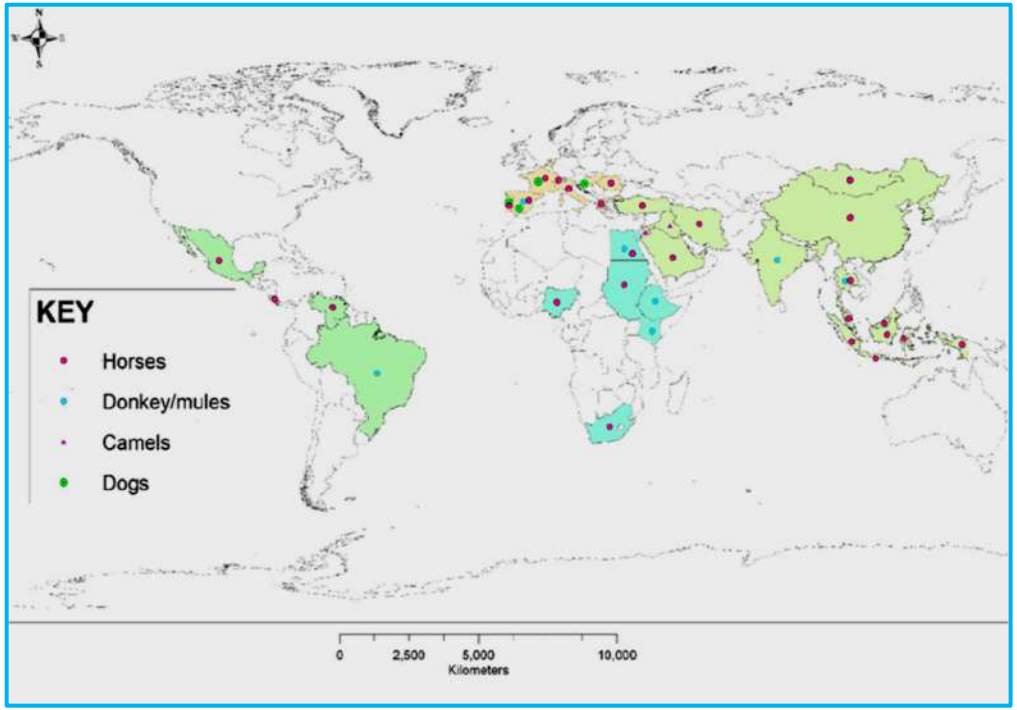


1. Transmission by tick bites

2. Vertical transmission

3. Transmission via blood transfusion

geographic distribution



Worldwide distribution of regions where equine piroplasms have been detected or reported across different hosts in the last ten years (2008–2018). Onyiche et al., 2019

The prevalence of ticks-carrying Pyriformis equine in eastern and southern Kazakhstan

Ticks and Tick-borne Diseases 12 (2021) 101817

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Tick distribution and detection of *Babesia* and *Theileria* species in Eastern and Southern Kazakhstan

Chunli Sang [a,1], Meihua Yang [b,1], Bin Xu [c,1], Guangyuan Liu [d], Yicheng Yang [a,e], Kenesbay Kairullayev [f], Otarbayev Bauyrzhan [f], Wurelihazi Hazihan [g], Sándor Hornok [h], Yuanzhi Wang [a,*]

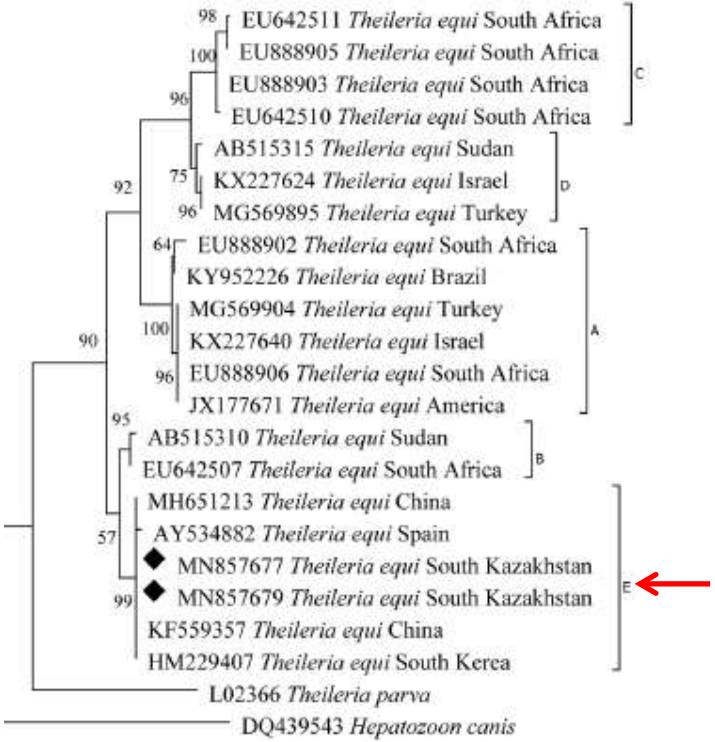
Collection site for tick samples in Kazakhstan

The positive rate of Pyriformis equine in tick samples from different regions of Kazakhstan

Table 1
Detection of Babesia spp. and Theileria spp. in ticks sampled from the east and south regions of Kazakhstan.

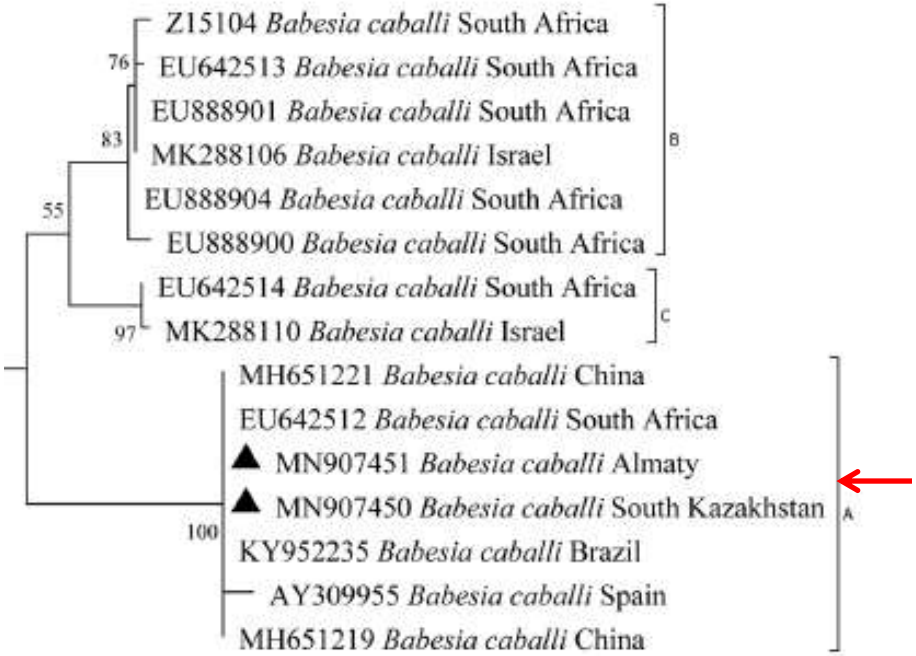
Oblast	District	Tick species	Origination	Number	*Babesia/Theileria* species, No. positive (%)
Almaty	Balkhash County (BH)	*Hyalomma asiaticum*	Off-host	47	*Babesia caballi*, 1 (2.13%)
		Haemaphysalis erinacei	Hedgehog	16	0
	Shagan City (SN)	*Rhipicephalus turanicus*	Goat	24	*Theileria annulata*, 5 (33.33%)
	Usharal County (UR)	*Hyalomma detritum*	Cattle	14	0
	Ushtobe County (UT)	*Dermacentor marginatus*	Horse	22	0
	Karabulak County (KL)	*Dermacentor marginatus*	Horse	10	*Babesia caballi*, 1 (10%) *Theileria equi*, 2 (20%)
East Kazakhstan	Zaysan County (ZS)	*Hyalomma detritum*	Cattle	48	0
		Dermacentor marginatus	Cattle	12	0
	Shyghys qazaqstan City (SQ)	*Dermacentor marginatus*	Cattle	15	0
South Kazakhstan	Chimkent City (CK)	*Dermacentor marginatus*	Cattle	36	*Babesia caballi*, 2 (5.56%)
			Horse	24	*Theileria equi*, 6 (25%)
		Argas persicus	off-host	102	0
	Saryagash County (SG)	*Dermacentor marginatus*	Cattle	159	*Babesia caballi*, 4 (2.30%) *Theileria annulata*, 4 (2.30%) *Babesia occultans*, 2 (1.45%)
		Dermacentor reticulatus	Horse	15	0
	Kazygurt County (KG)	*Rhipicephalus turanicus*	Shepherd dog	24	*Theileria annulata*, 4 (16.67%) *Theileria ovis*, 1 (4.17%)
Jambyl	Merki (MK)	*Dermacentor marginatus*	Cattle	20	0
	Lugovoy (LG)	*Hyalomma anatolicum*	Cattle	40	0
Kyzylorda	Aralsk County (AL)	*Hyalomma asiaticum*	Camel	70	0
Total 698					

Classification of 18S rRNA Genotypes of Theileria Equi



T. equi (MN857679 and MN857677) from South Kazakhstan and Almaty oblasts clustered into genotype E of this species, together with isolates from Spain (AY534882), China (MH651213 and KF559357) and South Korea (HM229407).

Classification of 18S rRNA Genotypes of Babesia



B. caballi from Almaty belongs to genotype A, which was also reported from China (MH651221), South Africa (EU642512), Spain (AY309955) and Brazil (KY952235).

Diagnostic methods recommended by the World Organization for Animal Health (OIE)

B 诊断方法

表 1 可用于诊断马梨形虫病的诊断方法及其目的

方法	目的					
	群体感染净化	个体感染净化	清除疾病策略的制定	确诊临床病例	感染率-监测	疫苗接种后免疫状况
抗原的鉴定						
显微镜检查	-	+	-	++	+	n/a
PCR	+++	+++	+++	+++	+++	n/a
免疫应答的检测						
IFAT	++	++	++	+++	++	n/a
c-ELISA	+++	+++	+++	+++	+++	n/a
CFT	+	+	+	+	+	n/a

VMRD cELISA diagnostic kit recommended by the World Organization for Animal Health (OIE)

vmrd Veterinary Medical Research & Development

FOR VETERINARY USE ONLY

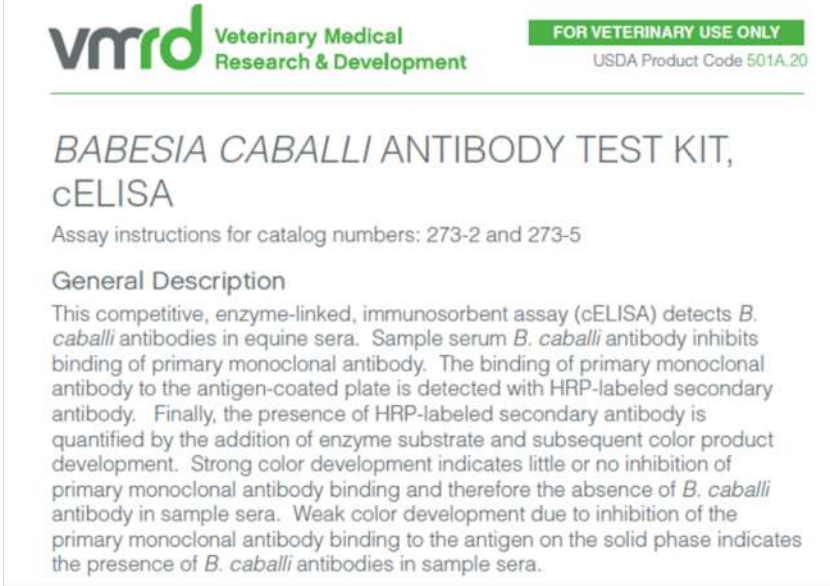
USDA Product Code 501B.20

BABESIA EQUI ANTIBODY TEST KIT, cELISA

Assay instructions for catalog numbers: 274-2 and 274-5

General Description

This competitive, enzyme-linked, immunosorbent assay (cELISA) detects *B. equi* antibodies in equine sera. Sample serum *B. equi* antibody inhibits binding of primary monoclonal antibody. The binding of primary monoclonal antibody to the antigen-coated plate is detected with HRP-labeled secondary antibody. Finally, the presence of HRP-labeled secondary antibody is quantified by the addition of enzyme substrate and subsequent color product development. Strong color development indicates little or no inhibition of primary monoclonal antibody binding and therefore the absence of *B. equi* antibody in sample sera. Weak color development due to inhibition of the primary monoclonal antibody binding to the antigen on the solid phase indicates the presence of *B. equi* antibodies in sample sera.



vmrd Veterinary Medical Research & Development

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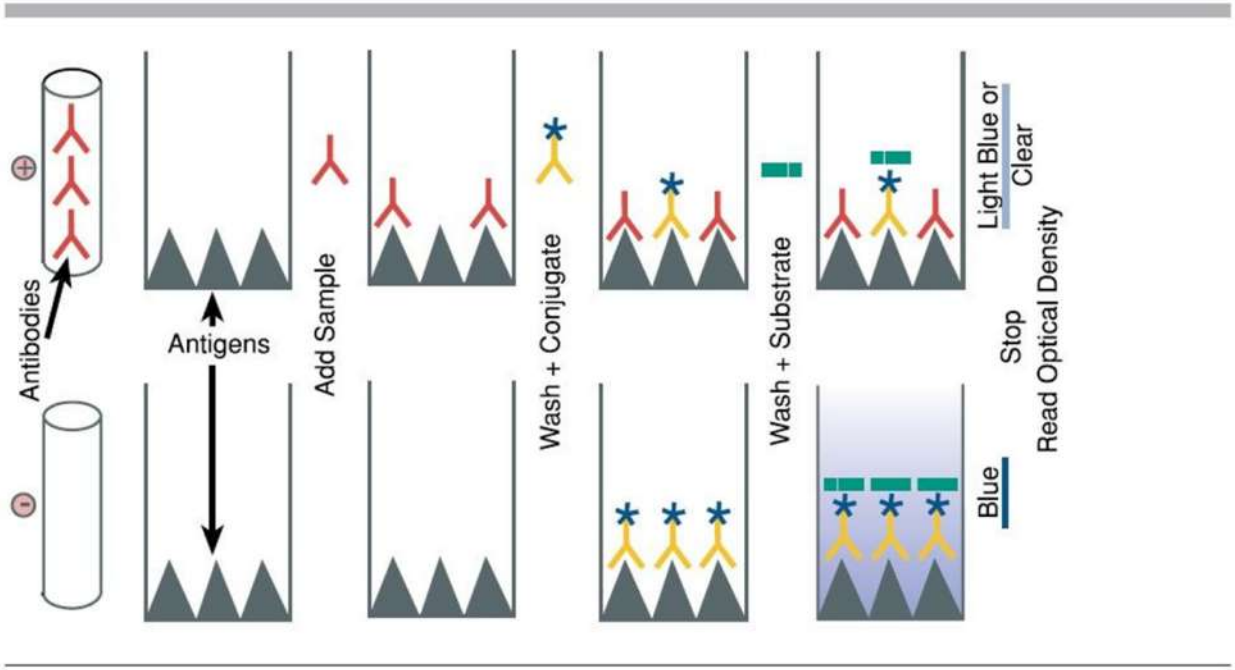
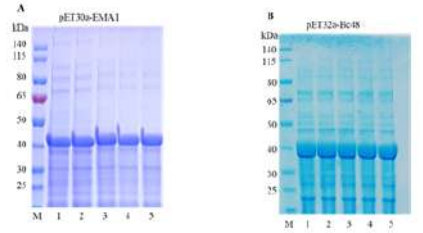
Competitive ELISA (cELISA) detection principle



High sensitivity, strong specificity

Preparation of monoclonal antibody and optimization of cELISA conditions

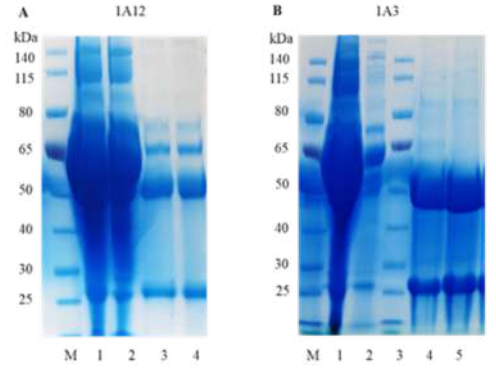


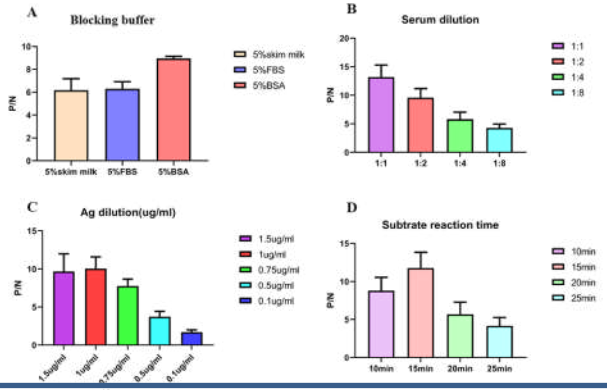
A: M: 蛋白分子质量标准; 1-5: pET-30a-EMA1蛋白纯化洗脱液

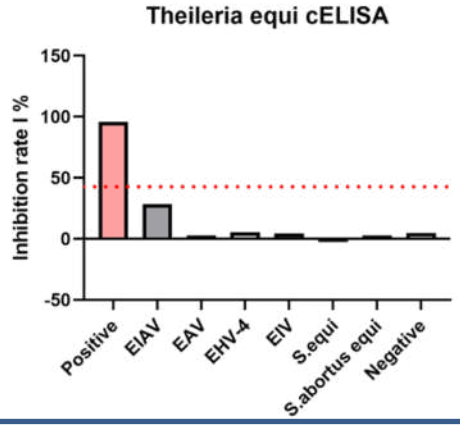
B: M: 蛋白分子质量标准; 1-5: pET-32a-Bc48蛋白纯化洗脱液

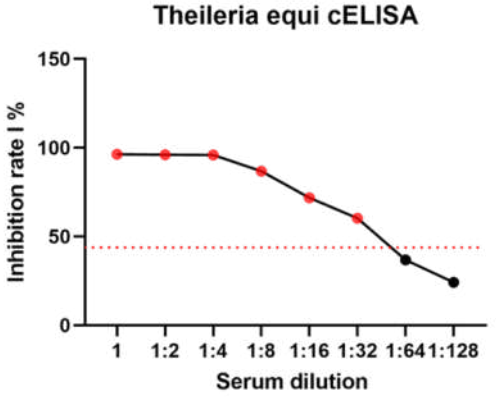
A

	EMA1		BC48	
	5H8	1A12	2D9	1A3
IgM	0.081	0.088	0.077	0.074
IgG1	1.623	1.466	1.473	1.524
IgG2a	0.078	0.074	0.074	0.072
IgG2b	0.084	0.083	0.105	0.08
IgG3	0.086	0.086	0.081	0.073
IgGA	0.083	0.081	0.085	0.071
Kappa	1.155	1.046	1.046	1.02
Lambda	0.09	0.085	0.085	0.08

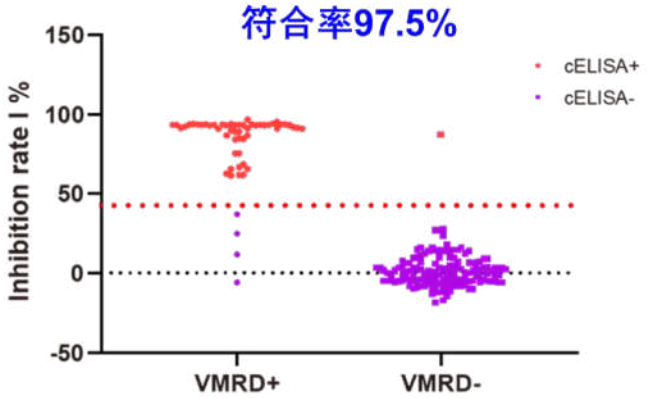








马梨形虫（马泰勒虫）抗体cELISA检测试剂盒



产品特点和优势

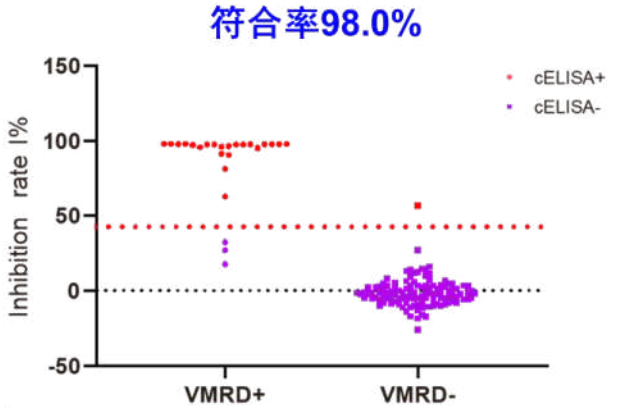
1. OIE标准，90分钟出结果；
2. 敏感性高，特异性强；
3. 检测成本低于国外产品；
4. 国际领先，国内独家。

服务马业！服务驴产业！

增值服务

可提供有资质实验室相关疾病检测服务

马梨形虫（驽巴贝斯虫）抗体cELISA检测试剂盒



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增值服务

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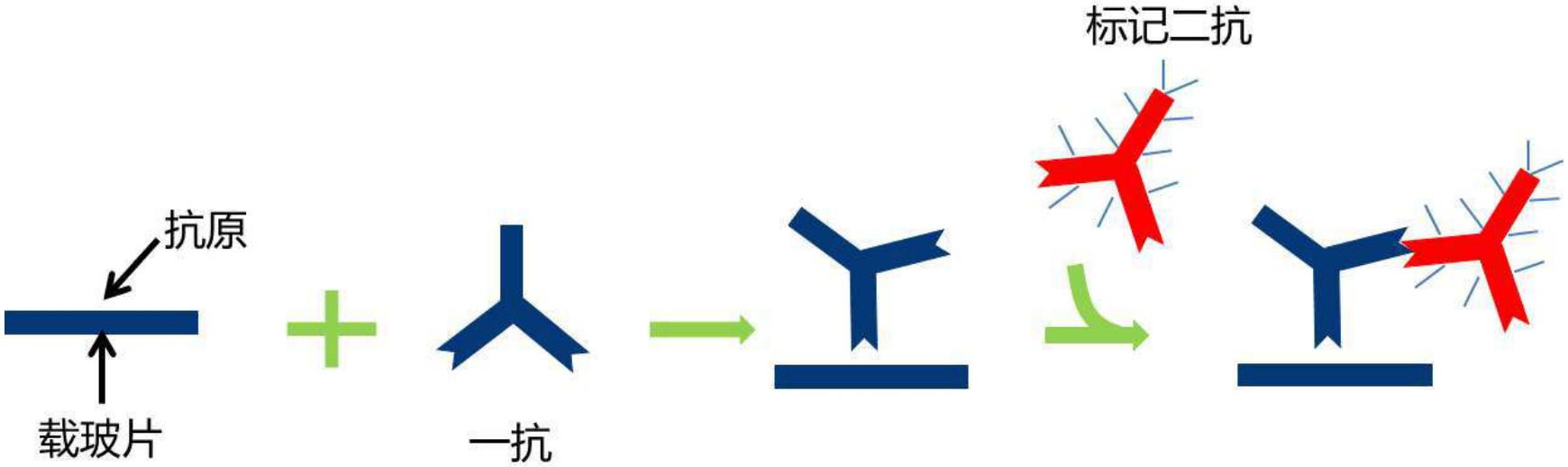
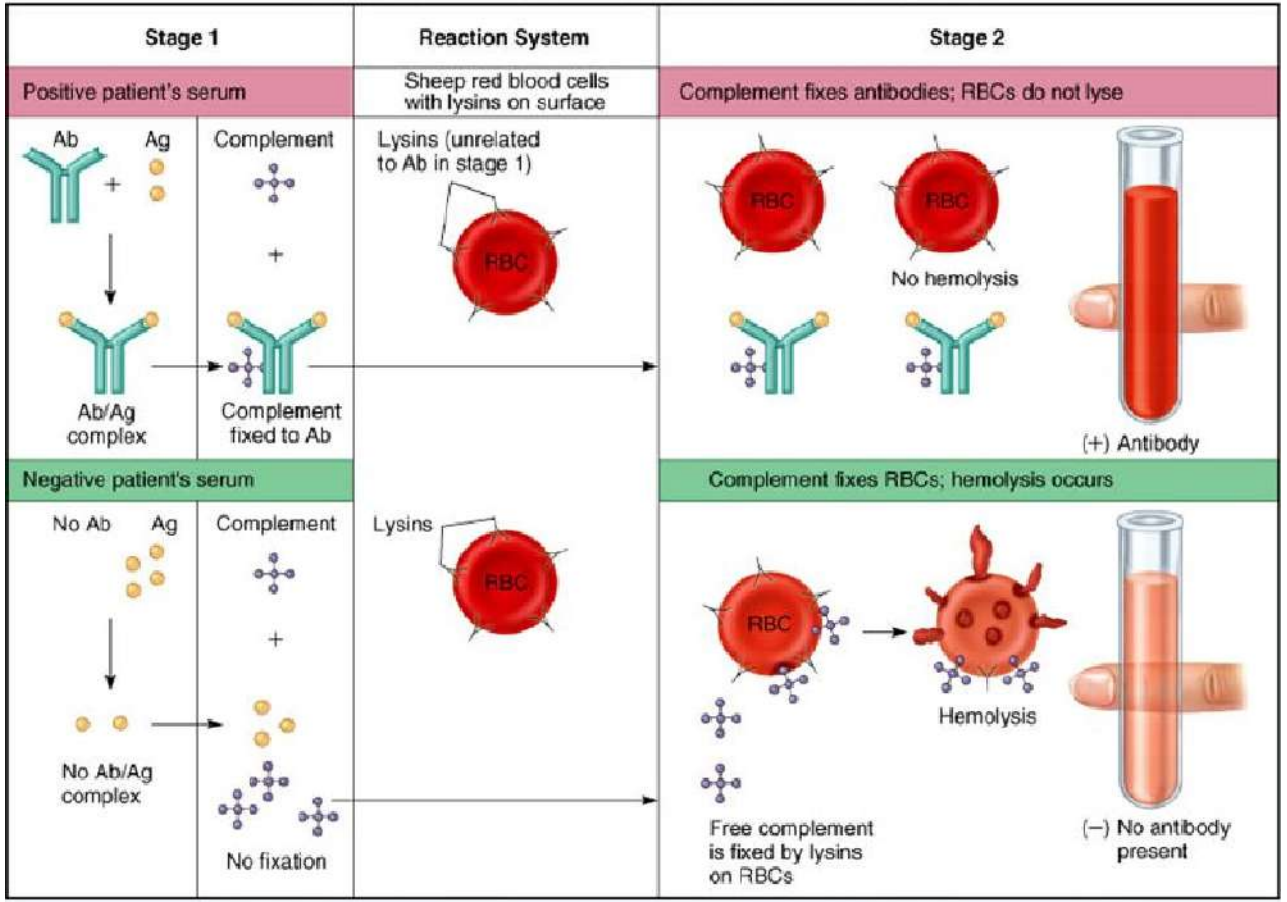
IFAT (indirect fluorescent antibody assay)

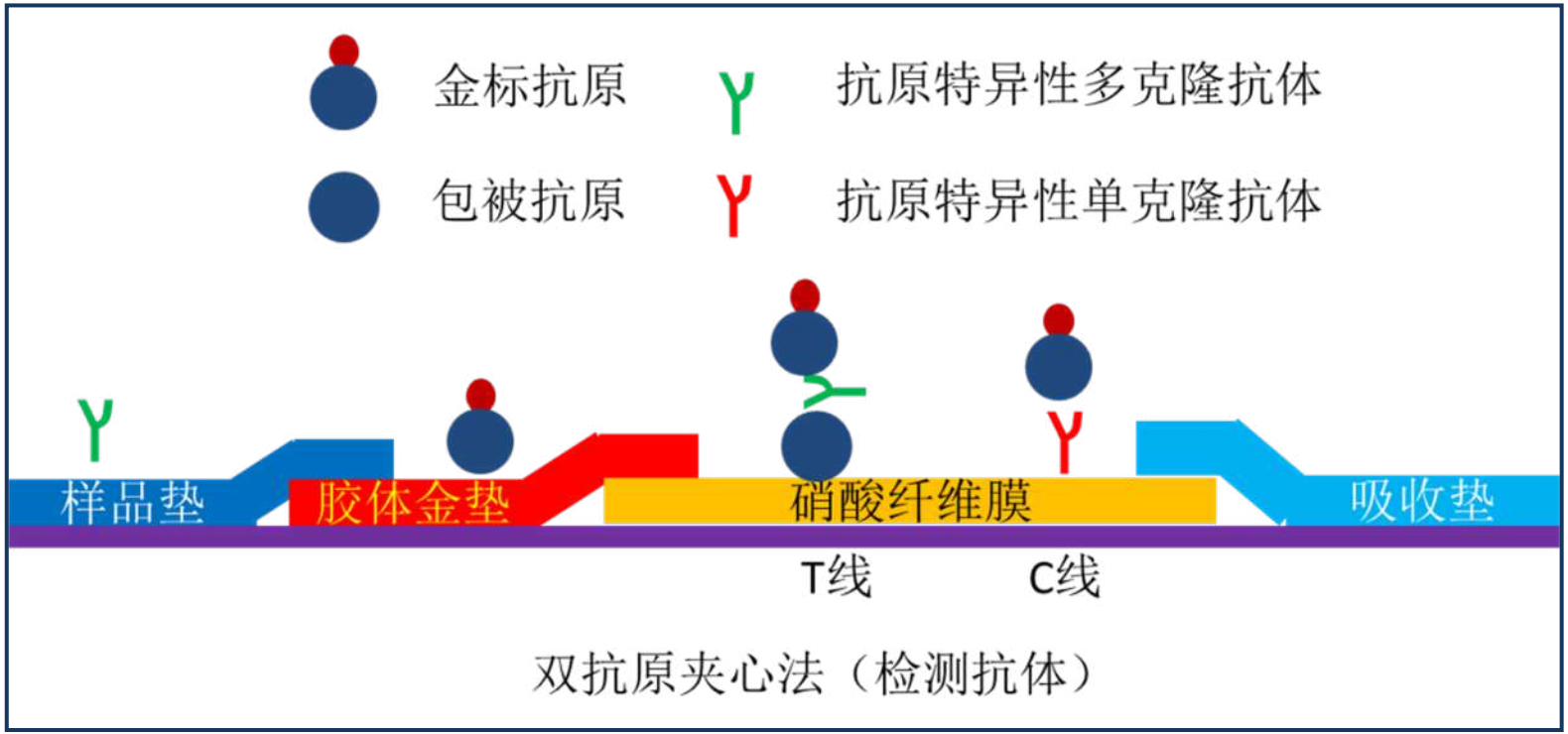


CFT

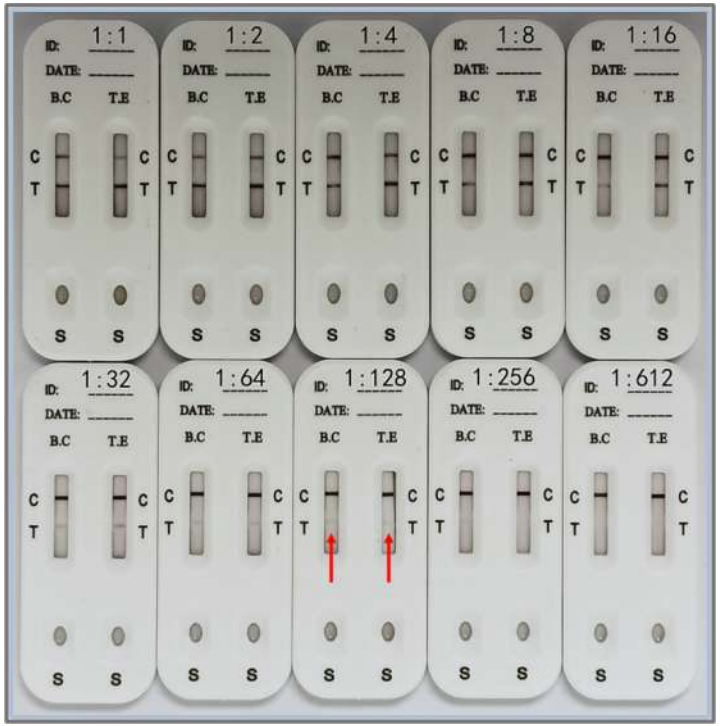
(Complement fixation test)



Double antigen sandwich colloidal gold detection principle

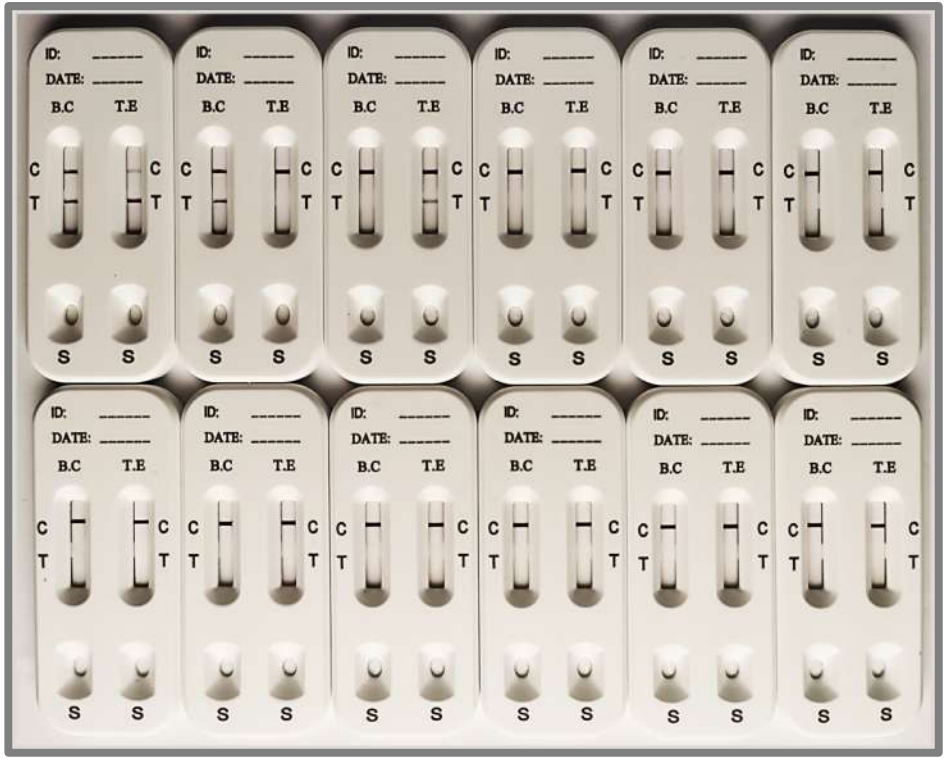


Colloidal gold sensitivity test



Both Theileria and Babesia positive sera can be detected at 1:128

Colloidal gold specific test



It does not react with the positive serum of common equine infectious diseases, mainly including: Escherichia coli, Salmonella abortus equine, Streptococcus equine disease, equine herpes virus type IV, equine arteritis virus, equine melioidosis, equine infectious anemia, and Ehrlich Trypanosoma

Verification of compliance rate with VMRD kit

Detection methods	VMRD-cELISA					
	T.equi			B.caballi		
	Positive(%)	Negative(%)	Row totals(%)	Positive(%)	Negative(%)	Row totals(%)
Test strip						
Positive	56(11.76)	8(1.68)	64(13.45)	23(4.83)	6(1.26)	29(6.09)
Negative	9(1.89)	403(84.66)	412(86.55)	4(0.84)	443(93.07)	447(93.91)
Column totals	65(13.66)	411(86.34)	476(100)	27(5.67)	449(94.33)	476(100)

Through the detection and calculation of 476 clinical samples from different regions, it is shown that the coincidence rate for Theileria is 96.43%, and the coincidence rate of Babesia

马梨形虫（马泰勒虫）抗体胶体金检测卡

产品特点和优势

1.一滴血清检测，现场10分钟出结果；
2．敏感性高，可用于初筛；
3．国内外无同类产品；
4．国际领先，国内独家。

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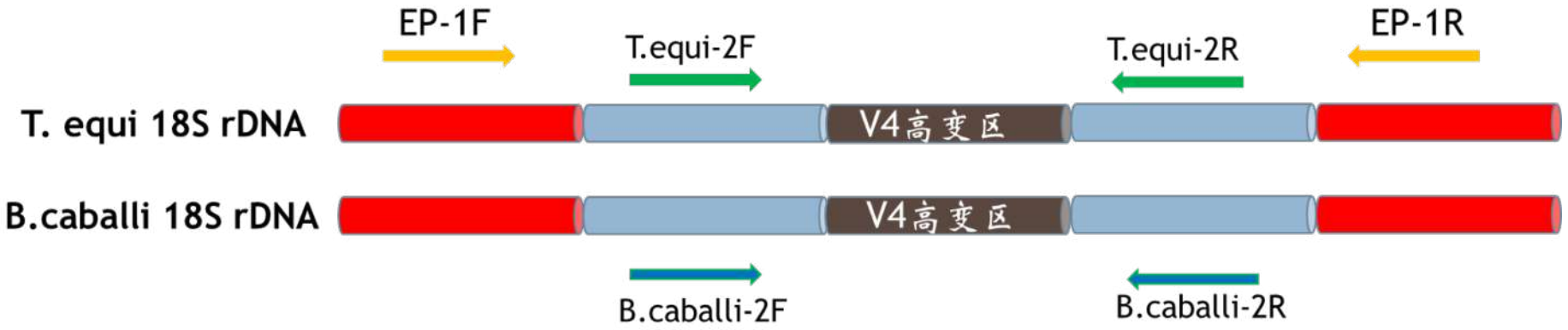
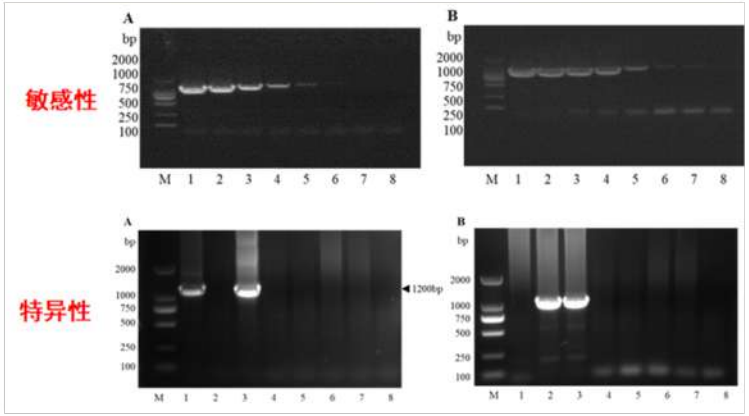
Nested PCR primer design



马梨形虫（马泰勒虫和弩巴贝斯虫）PCR检测试剂盒



产品特点和优势

1．OIE和行业双重标准；
2．敏感度高，特异性强；
3．可同时检测两种病原；
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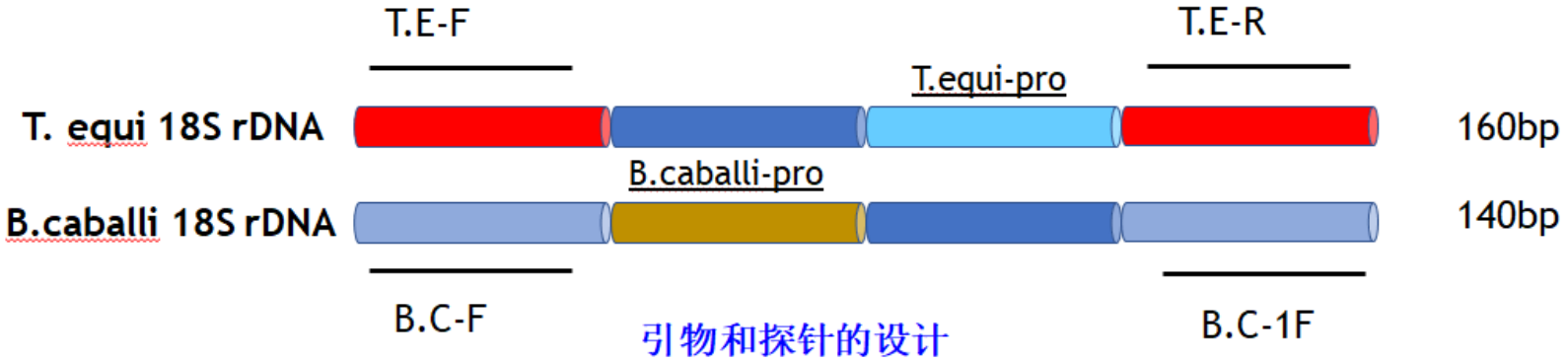
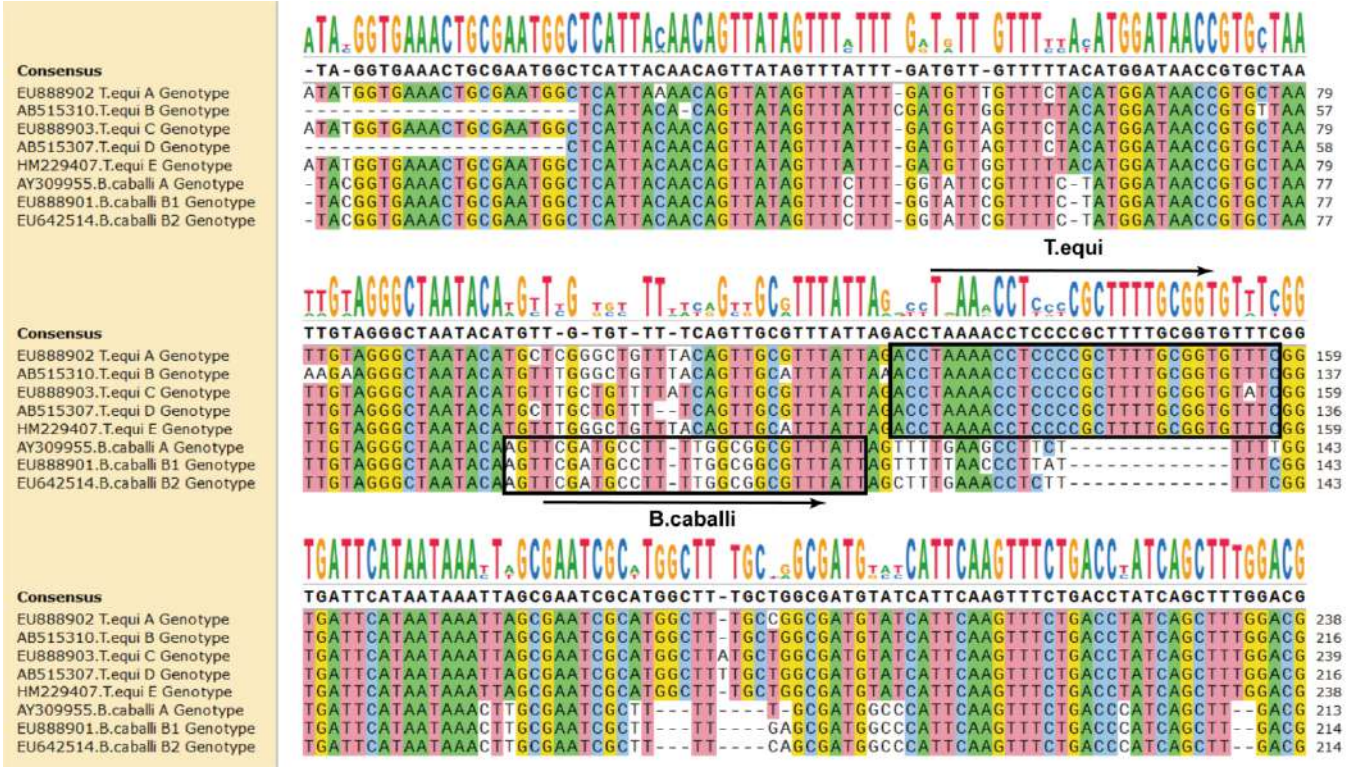
Double fluorescence quantitative PCR



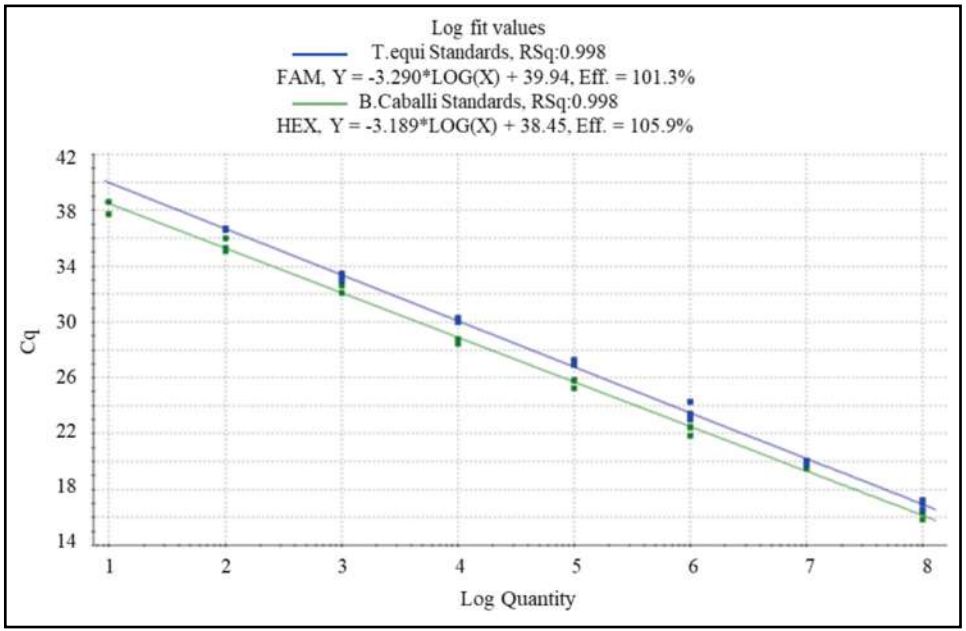
反应过程	温度(℃)	时间	循环数(Cycles)
预变性	95	1min	1
变性	95	15s	40
退火/延伸	60	30s	40

Design of Double Fluorescence Quantitative PCR Primers



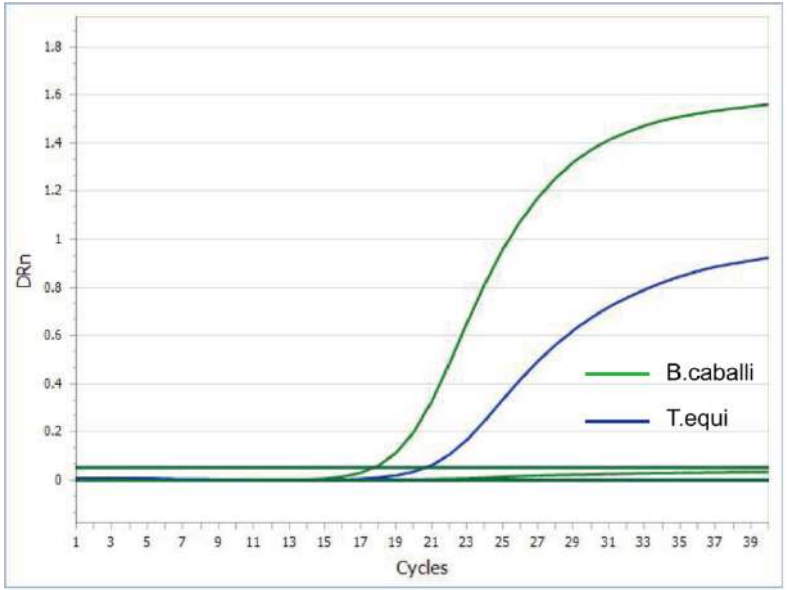
Primers and probes were designed for the conserved regions of the 18S rRNA V4 regions of different genotypes of Theileria and Babesia

Establishment of Standard Curve of Double Fluorescence Quantitative PCR



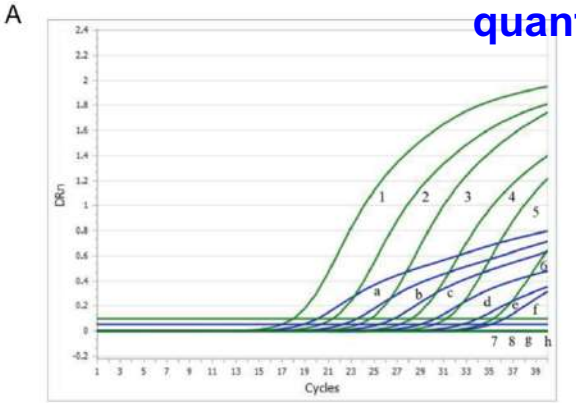
The correlation coefficient R2 is 0.99, and the amplification efficiency is 101.3% and 105.9%, respectively

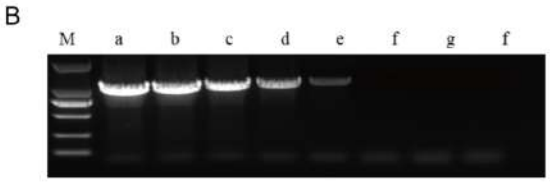
Specificity test of dual fluorescence quantitative PCR

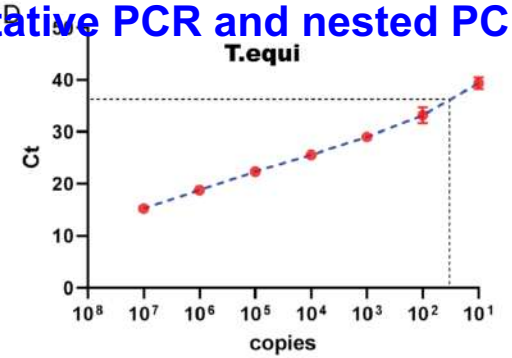


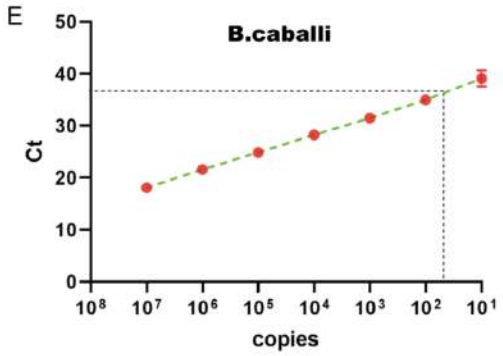
It only reacts with positive samples of Theileria equi and Babesia, and does not react with other pathogens of equine infectious diseases, equine herpes virus, equine infectious anemia virus, equine blandular disease and equine abortion salmonella positive DNA

Comparison of the sensitivity of dual fluorescence quantitative PCR and nested PCR









The sensitivity of nested PCR is 1 x 10^2 copies/μL

The sensitivity of dual fluorescence quantitative PCR is

1 × 10^3 copies/μL

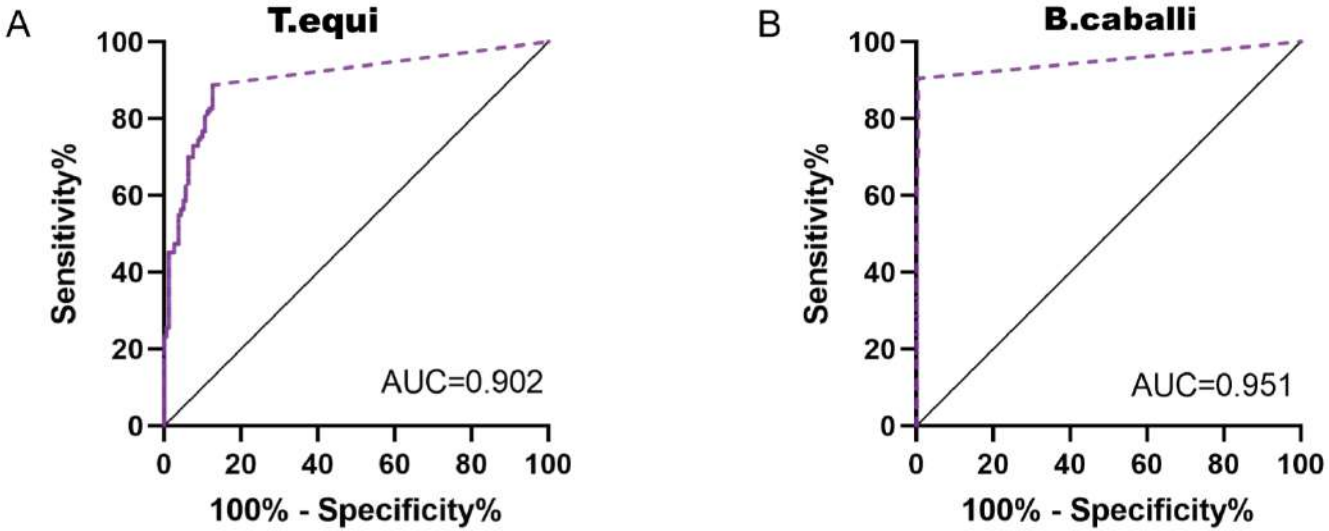
Repeatability of double fluorescence quantitative PCR

Pathogens	Concern of template (copies)	Intra-coefficient of variation		Inter-coefficient of variation	
		$\bar{X}$±SD	CV(%)	$\bar{X}$±SD	CV(%)
T.equi	10^8	16.92±0.28	1.69	17.01±0.03	0.19
	10^7	19.94±0.13	0.64	20.31±0.35	1.75
	10^6	23.56±0.53	2.28	23.88±0.24	1.01
	10^5	27.16±0.17	0.64	26.88±0.13	0.51
	10^4	30.12±0.13	0.45	30.30±0.32	1.06
B.caballi	10^8	16.17±0.22	1.37	17.23±0.24	1.39
	10^7	19.48±0.01	0.41	20.69±0.08	0.40
	10^6	22.27±0.27	1.22	24.14±0.14	0.58
	10^5	25.64±0.26	1.03	26.73±0.13	0.50
	10^4	28.62±0.14	0.51	30.56±0.34	1.14

The CV values of the coefficient of variation of the repeatability within and between batches of theileria were 0.45-2.28% and 0.19-1.75%, respectively

The CV values of the intra-batch and inter-batch repeatability of Babesia are 0.41-1.37% and

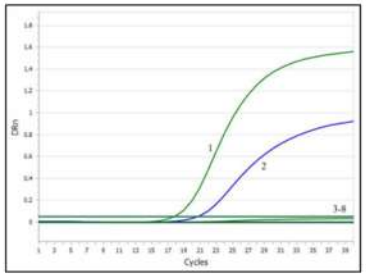
The coincidence rate of dual fluorescence quantitative PCR and nested PCR and sequencing



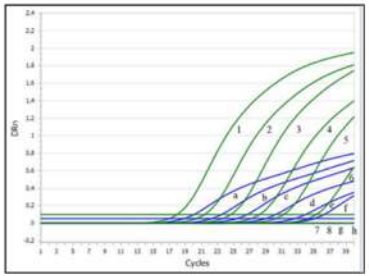
Through DNA testing of 500 clinical samples from different regions, The ROC area is 0.902 and 0.951, P-value is less than 0.05, and the coincidence rate is 99.85% and 98.6%, respectively

马梨形虫（马泰勒虫和弩巴贝斯虫）双重荧光定量PCR检测试剂盒

特异性强



敏感度高



产品特点和优势

1．快速，60分钟出结果；
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马梨形虫病检测系列产品

产品名称	检测样品	备注
马梨形虫（马泰勒虫）竞争ELISA抗体检测试剂盒	马、驴血清	检测马梨形虫抗体，优于OIE方法
马梨形虫（弩巴贝斯虫）竞争ELISA抗体检测试剂盒	马、驴血清	检测马梨形虫抗体，优于OIE方法
马梨形虫（马泰勒虫和弩巴贝斯虫）抗体胶体金检测卡	马、驴血清	一滴血清检测，现场10分钟出结果，用于初筛
马梨形虫（马泰勒虫）抗体胶体金检测卡	马、驴血清	一滴血清检测，现场10分钟出结果，用于初筛
马梨形虫（弩巴贝斯虫）抗体胶体金检测卡	马、驴血清	一滴血清检测，现场10分钟出结果，用于初筛
马梨形虫（马泰勒虫和弩巴贝斯虫）PCR检测试剂盒	马、驴抗凝血	核酸检测，中国出入境检疫行业标准
马梨形虫（马泰勒虫和弩巴贝斯虫）双重荧光PCR检测试剂盒	马、驴抗凝血	核酸检测，优于OIE方法

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闫东 电话：13304817269

技术服务：中国农业科学院哈尔滨兽医研究所
杜承 电话：15134556332

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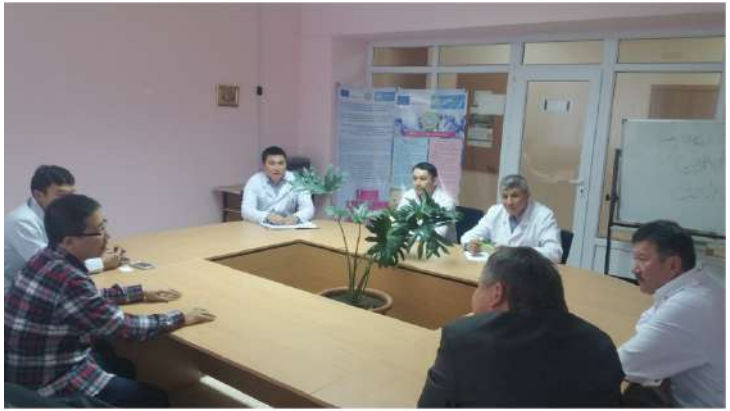
Thank you

国家重点研发计划

Visit of Severin Agricultural University of Kazakhstan in 2015



Welcome to Harbin Veterinary Research Institute to visit and study!

спасиб **рахмет**