

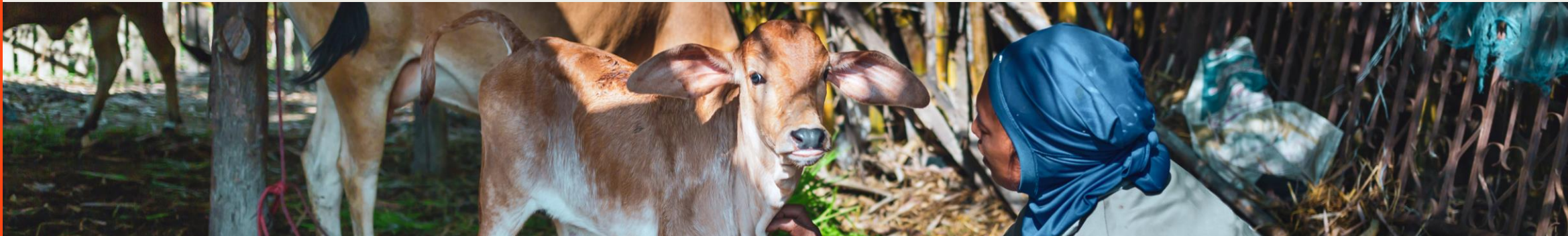


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Founded in 1924

中华人民共和国农业农村部
Ministry of Agriculture and Rural Affairs of the People's Republic of China

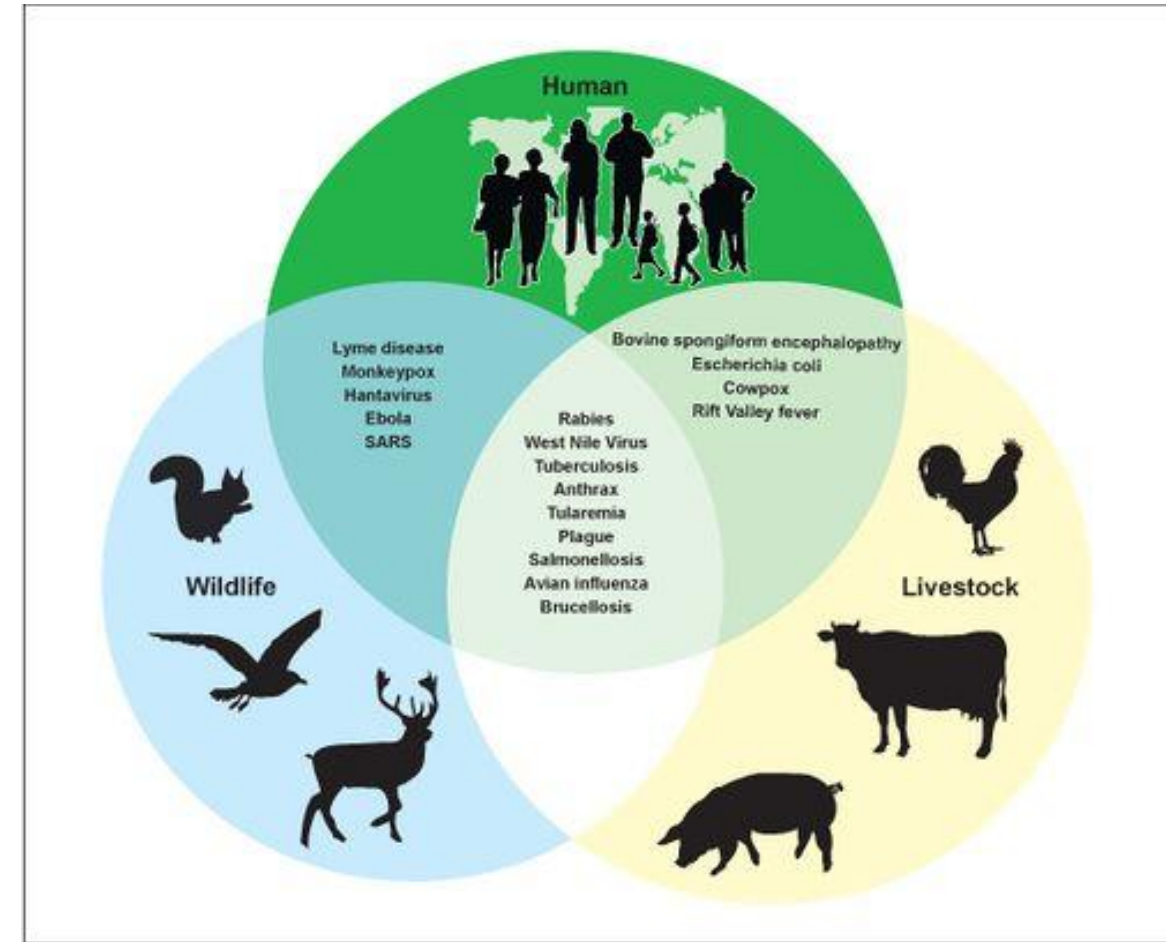
Rapid TB Diagnosis in Cynomolgus Macaques: Leveraging Xpert MTB/RIF Ultra Assay for Enhanced One Health Outcomes

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Tuberculosis in Macaques: A One Health Concern

- TB caused by the *Mycobacterium tuberculosis* complex (MTBC) affects both humans and animal.
- TB contributes to 1.6 million deaths annually worldwide.
- Transmission posed risk to public health, animal health, and ecosystem



Source: QAO analysis of UBSIS data (data); Art Explosion (Images)

https://www.physio-pedia.com/Zoonotic_Diseases

Tuberculosis in Cynomolgus Macaques

- Cynomolgus macaques (*Macaca fascicularis*) are important models for TB research and often cohabit regions with humans and animals.
- Managing TB in macaques is crucial for preventing zoonotic transmission and ensuring animal welfare.





Conventional TB Diagnosis in Macaques: Limitations

Chest Radiographs

- Can miss early-stage or asymptomatic TB cases.
- Limited sensitivity in detecting TB in macaques.

Tuberculin Skin Test (TST)

- Standard TB test used in humans, also applied to macaques.
- Prone to false-negative or false-positive results.
- Cross-reactivity with non-tuberculous mycobacteria (NTM) is a common issue.

Contamination Issues in Culture Methods

- Inadequate decontamination during the processing of biological samples
- Overgrowth of Non-Tuberculous Bacteria or Fungi
- Long Incubation Time

Xpert MTB/RIF Ultra Assay: A Molecular Approach

- The Xpert MTB/RIF Ultra assay is a rapid molecular diagnostic tool widely used in human TB diagnosis.
- It detects **MTBC DNA and rifampicin resistance** directly from clinical samples.
- High sensitivity and specificity make it suitable for detecting TB in cynomolgus macaques.

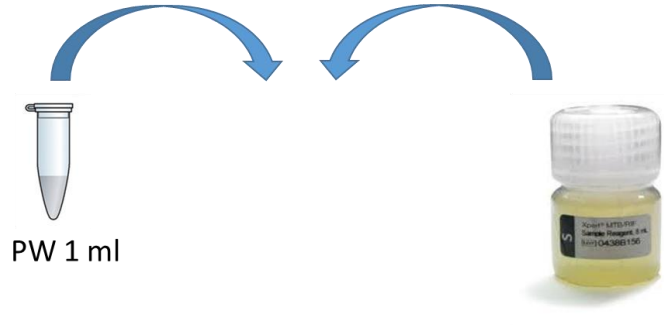


"6-colour optics"



Xpert MTB/RIF Ultra Assay: A Molecular Approach

1. PW liquefaction and inactivation with 2:1 sample reagent



2. Incubate for 15 minutes at room temperature and transfer of 2 ml material to Xpert Ultra cartridge



3. Insert cartridge platform and run. Results in <80 minutes



- ✓ **MTBC (~16 CFU/ml) and rifampicin resistance (~105.4 CFU/ml) detection**
- ✓ **Real-time PCR / Complete closed automate system**
- ✓ **Recommended for smear-negative**



Applying Xpert MTB/RIF Ultra in Macaques



- Krabok-Koo Wildlife Breeding Center (Nationwide)
- Captive cynomolgus macaques, in total 316 individuals distributed in 42 group cages
- PW involved collecting samples from the upper respiratory tract, specifically the oropharyngeal (mouth and throat) and tracheal areas



Study Design: Testing in Cynomolgus Macaques

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0, 3, 6, 12 months (follow-up evaluation)

PW samples



Xpert MTB/RIF Ultra



Every 3 months

Direct smear (AFB)
Culture



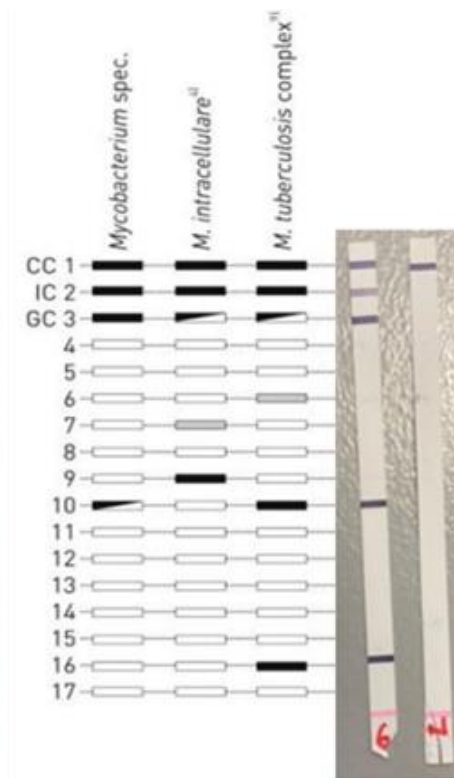
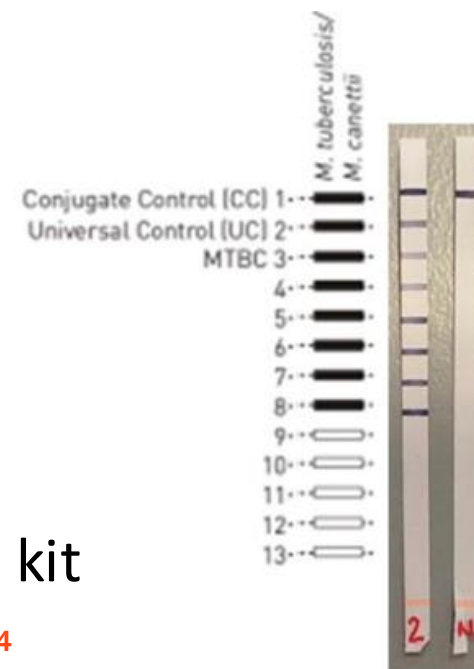
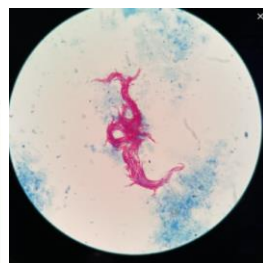
Culture positive (AFB+)



LPA (Hain Life Science, Germany)

GenoType MTBC or/and GenoType Mycobacterium CM kit

Remarks. PW, BS, body weight recording, TST, chest radiographs, other necessary physical examinations



Mf no	Cage no	PW samples									Tissue autopsy
		Month 0 N=12 (3.8%)		Month 3 +4		Month 6 +1		Month 9	Month 12 +1		Alive/death (month) 5
		Xpert (Ct)	AFB, Culture	Xpert (Ct)	AFB, Culture	Xpert (Ct)	AFB, Culture	AFB, Culture	Xpert (Ct)	AFB, Culture	AFB, Culture
46	C1/1	<u>+(27.6)^T</u>	AFB+, Min	-	AFB+, cont	ND	ND	ND	ND	ND	Dead (7 MO), ND
49	C1/1	-	AFB+, Mfo	<u>+(23.7)^L</u>	NG	-	NG	NG	-	NG	Alive (9 MO)
55	C2/1	-	NG	<u>+(26.1)^L</u>	NG	-	NG	NG	-	NG	Alive (9 MO)
91	cage 5/1	<u>+(16.5)^L</u>	NG	ND	ND	ND	ND	ND	ND	ND	Dead (1 MO), ND
96	cage 5/1	<u>+(24.2)^T</u>	NG	ND	NG	-	NG	NG	-	NG	Alive (15 MO)
99	cage 5/1	-	NG	<u>+(19.9)^L</u>	NG	<u>+(17.2)^L</u>	<u>AFB+, Mtb</u>	ND	ND	ND	Dead (4 MO), Lung&Liver found Mtb
100	cage 5/1	-	NG	-	AFB-, cont	-	NG	NG	<u>+(17.0)^L</u>	NG	Alive (0 MO)
102	cage 5/1	<u>+(24.6)^T</u>	<u>AFB+, Mtb</u>	-	NG	-	NG	NG	-	NG	Alive (15 MO)
											Dead (11 MO),
<p>✓ 100% sensitivity, detecting all culture-confirmed TB cases.</p> <p>✓ 13.0% of TST-positive macaques and 13.3% with positive chest radiographs tested positive with Xpert MTB/RIF Ultra</p> <p>The Xpert MTB/RIF Ultra assay proved more sensitive and specific than TST and chest radiographs, detecting MTBC DNA in PW samples</p>											
292	Q1	-	NG	-	NG	<u>+(26.1)^T</u>	NG	NG	-	NG	Alive (7 MO)
293	Q1	-	NG	<u>+(23.0)^T</u>	NG	<u>+(16.4)^M</u>	NG	ND	ND	ND	Dead (4 MO), Lung found Mtb
298	Q3	<u>+(29.3)^T</u>	AFB+, cont	-	NG	-	NG	NG	-	NG	Alive (13 MO)

PW samples were more effective, with 5.1% positivity, compared to 0% for buccal swabs

		No. (%) of BS result	
		Negative	Positive
No. (%) of PW result	Negative	94 (94.9)	0 (0.0)
	Positive	5 (5.1)	0 (0.0)

Table 2. Comparative Analysis of Xpert MTB/RIF Ultra Results between PW and BS Samples. $P=0.063$ by McNemar's test.

- BS specimens are oral sampling and one of the common sites of MTBC shedding, making it a likely less invasive specimen collection method
- 3/5 (no. 99, 103, and 293) positive macaques died in several months later.



Impact of Rapid Diagnosis combined with TB Control strategy

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Gang cage

individual cage

Gang cage

individual cage

C1/1																											
month 0	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	47	48	49	46								
month 3	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	47	48	49									
month 6	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	47	48										
month 12	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	47	48										

i01_c1/1	i02_c1/1
46	
46	49
46	49
	49

cage 5/2
month 0 200 199
month 3 200
month 6 200
month 12 200

i16_cage 5/2
199
199
199
199

cage 4/5									
month 0	193	194	196	197	198	195			
month 3	193	194	196	197	198				
month 6	193	194	196	197	198				
month 12	193	194	196	197	198				

i03_C4/5
195
195
195
195

Q3
month 0 299 300 298
month 3 299 300
month 6 299 300
month 12 299 300

i17_Q3
298
298
298
298

cage 5/1														
month 0	92	93	94	95	97	98	104	100	101	99	91	96	102*	103 105
month 3	92	93	94	95	97	98	104	100	101	99				
month 6	92	93	94	95	97	98	104	100	101					
month 12	92	93	94	95	97	98	104	100						

i04_C5/1	i05_C5/1	i06_C5/1	i07_C5/1	i08_C5/1	i09_C5/1	i10_C5/1
91	96	102*	103	105		
91	96	102	103	105	99	
	96	102	103*	105	99*	
	96	102	103*	105	99*	100

C2/1																											
month 0	50	53	54	56	58	59	60	61	62	63	64	66	67	68	69	70	72	52	57	65	51	71	53				
month 3	50	53	54	56	58	59	60	61	62	63	64	66	67	68	69	70	72	52	57	65	51	71	53				
month 6	50	53	54	56	58	59	60	61	62	63	64	66	67	68	69	70	72	52	57	65	51	71	53				
month 12	50	53	54	56	58	59	60	61	62	63	64	66	67	68	69	70	72	52	57	65	51	71	53				

i18_Q2/1
55
55
55
55

Q1
month 0 294 292 293
month 3 294 292 293
month 6 294 292
month 12 294

i11_Q1	i12_Q1
293	
293	292
293*	292

cage 3/5									
month 0	169	171	172	173	174	175	170	168	
month 3	169	171	172	173	174	175	170	168	
month 6	169	171	172	173	174	175	170	168	
month 12	169	171	172	173	174	175	170		

cage 2/2									
month 0	114	115	116	117	118	119	120		
month 3	114	115	116	117	118	119			
month 6	114	115	116	117	118	119			
month 12	114	115	116	117	118	119			

i13_cage 2/2
120
120
120
120

C4/1									
month 0	83	84	85	88	87	89	90	86	
month 3	83	84	85	88	87	89	90	86	
month 6	83	84	85	88	87	89	90	86	
month 12	83	84	85	88	87	89	90	86	

N9									
month 0	262	263	265	267	268	269	270	264	266
month 3	262	263	265	267	268	269	270		
month 6	262	263	265	267	268	269	270		
month 12	262	263	265	267	268	269			

i14_N9	i15_N9
264	266
264	266
264	266
264	266

Negative

Positive

relocate

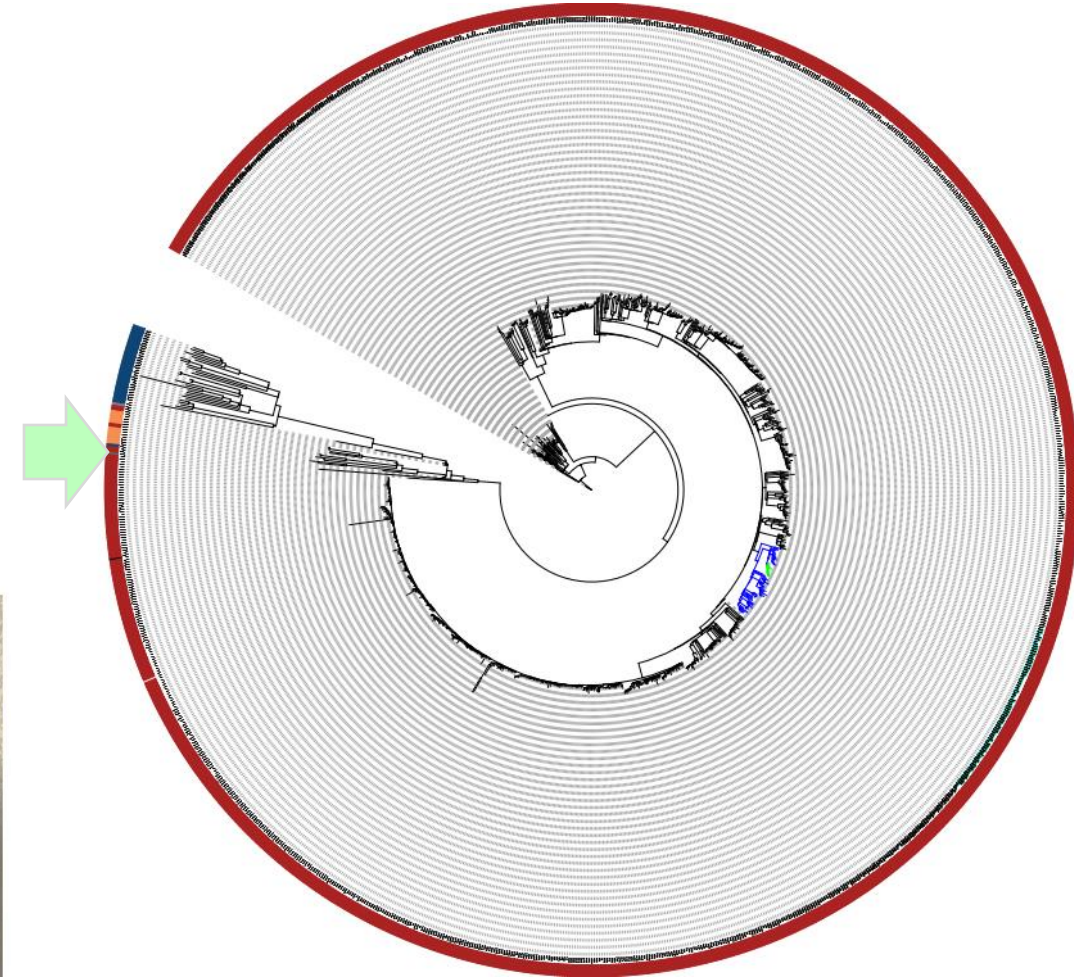
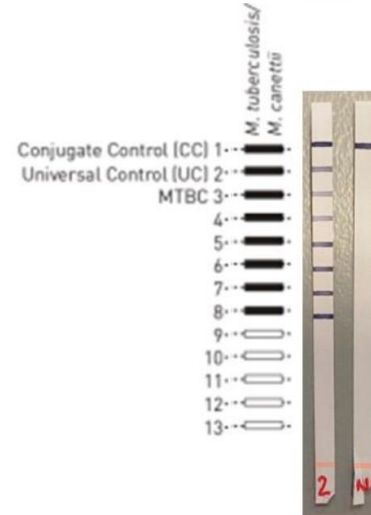
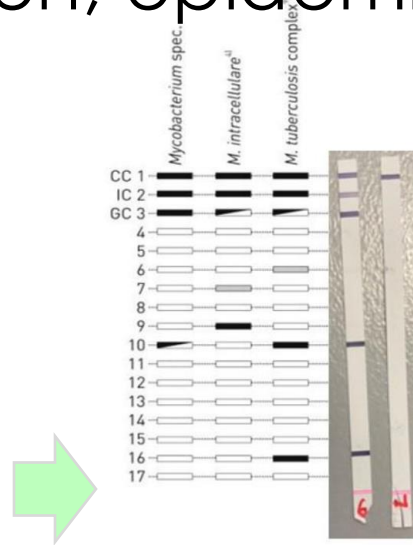
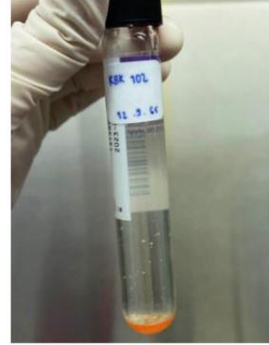
Dead

*positive both in Xpert MTB/RIF
Ultra assay and in culture

Conclusion: A Tool for Better TB Management

- The Xpert MTB/RIF Ultra assay is a highly sensitive, rapid diagnostic tool for detecting TB in cynomolgus macaques.
- The Xpert MTB/RIF Ultra assay can be highly suitable for use in reference laboratories to confirm TB disease and effectively interrupt TB transmission

Mtb in animal: diagnosis (conventional, molecular; commercial/in-house), species identification, epidemiological study





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Thank you

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