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JOURNAL OF

MOLECULAR DIAGNOSTICS AND THERAPY

Volume 9 Number 1 January 2017

P35 HE ×200

Figure P35 The positive results of TCT in patients with malignant peritoneal effusion HE ×200

ISSN 1674-6929

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分子诊断与治疗杂志

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TianYi Yofus Technology Co., Ltd

分子诊断与治疗杂志

2017 1 9 1

Bimonthly Volume 9 Number 1 January 2017

COMMENTS

ORIGINAL ARTICLES

Bimonthly Volume 9 Number 1 January 2017

REVIEWS

6

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[ABSTRACT] During the past ten years, circulating tumor cells (CTCs) have received enormous attention as potential research direction, because of the potential value in the early diagnosis of cancer, prediction of clinical prognosis, evaluation of therapeutic efficiency, development of targeted drugs, and personalized medicine of malignant cancer. Circulating tumor cells (CTCs) have been regarded as minimally invasive and promising diagnostic and prognostic biomarkers for patients with metastatic tumors. Multiple highly sensitive approaches and devices have been developed to separate and enrich CTCs in the blood. We have summarized 6 kinds of separation and enrichment technologies of CTCs with the opportunities and challenges faced during the development of these systems, this article also provides reference frame for an optimized separation and enrichment technology and highlight some directions for the precision medicine of cancer treatment.

[KEY WORDS] Circulating tumor cells (CTCs); Precision medicine; Separation and enrichment

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Ƀ NanoVelcro cell-affinity substrates CTC
ɞ ا Aptamer ɞ
microfluidics chip technology ɞת Ꮒ
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[2 12] ɟ

1 CTCs

1.1 NanoVelcro cell-affinity substrates CTC

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VerIFAST CTCs
[26-28] Massachusetts General Hospi-
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[2, 36] 3D [37]
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met all inclusion criteria and were included in this meta-analysis. Our results revealed that the 677C T polymorphism might increase the risk of CVD (Allele T C: OR=1.39, 95%CI=1.17, 1.65, =0.000 2; TT CC: OR=1.57, 95%CI=1.16, 2.12, =0.003; TC CC: OR=1.69, 95%CI=1.26, 2.27, =0.000 4; TT+TC CC: OR=1.53, 95% CI=1.21, 1.94, =0.000 5; TT TC + CC: OR=1.37, 95% CI=1.08, 1.73, =0.01; respectively). Further subgroup analysis by variety of disease and age range suggested that the 677C T polymorphism was associated with an elevated risk for CVD among coronary heart disease (TT TC+CC: OR=1.38, 95%CI=1.17, 1.62, =0.000 1), myocardial infarction (Allele T C: OR=1.71, 95%CI=1.37, 2.13, 0.000 01), with age range of less than 50 years (TT CC: OR=2.23, 95%CI=1.58, 3.17, TC ╔ 8 U ή Л

Diseases, Cardiovascular[Text Word]) OR Cardiovascular Diseases[Text Word])) AND (((((((Methylenetetrahydrofolate Reductase[Text Word]) OR Methylene-THF Reductase[Text Word]) OR 5,10-Methylenetetrahydrofolate Reductase[Text Word]) OR Methylene Tetrahydrofolate Reductase[Text Word]) OR Tetrahydrofolate Reductase, Methylene[Text Word]) OR MTHFR[Text Word]) OR NADPH[Text Word]) ɟ

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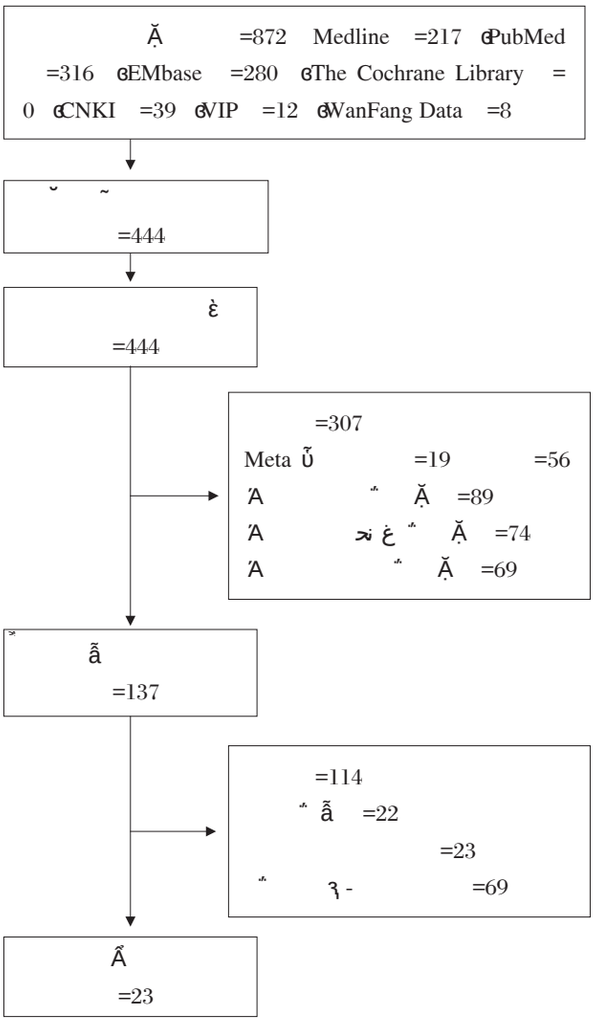

1 Meta

Figure 1 Flow chart of the study selection process and specific reasons for exclusion from the meta-analysis

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Juan 2015 [10]	نص Ɔ		373/391	58/53	♥ ה ב	
Javed 2012 [11]	ẋ Ж		377/393	63.1±10.9/61.0±10.5	♥ ה ב	
Alexandre 2007 [12]			91/36	60.3±12/60.4±12	ơ	
Soujatya 2010 [13]	○ Z		217/255	42.60/40.53	ơ	
Angeline 2009 [14]	○ ⊥		120/100	–		
Nevin 2008 [15]	–		78/100	57.2±10.0/57.5±11.1	♥ ה ב	
Nevin 2008 [15]	–		100/100	58.3±9.9/57.5±11.1	ơ	
Jones 2005 [16]	سھ		271/282	64.0±9.5/69.5±7.6	ơ	
Angeline 2004 [17]	○ ⊥		52/20	–		
Klaus 2004 [18]	ж		2121/617	66.8±12.8/60.8±12.4	ơ	
Irena 2003 [19]	ẋ ئشЖ		247/298	–		6
Lakhdar 2009 [20]			352/390	58.0±11.5/57.3±7.6		
Vasisht 2002 [21]	○ ←		141/55	52±10/50±11		
Anne 2001 [22]	ه ж		110/185	56/55		
Sadi 2001 [23]	ھ ę		96/100	38±7/37±5		
S.Friso 2002 [24]	ẋ		302/168	60.6±9.3/58.2±12.8		
Hamdia 2014 [25]	بج Ш		30/15	45.5±4.8/43.2±4.3		
Prithiksha 2015 [26]	⊥ ○ ж		106/100	37.6/37.5		
Dhouha 2014 [27]			100/200	46.92±7.62/31±9.6		
Andrzej 2001 [28]	–		161/211	43.6±4.7/43.7±5.6		
2007 [29]	Ψ ه		87/73	71.9±13.2/67.8±13.6		
2010 [30]	Ψ ه		114/31	77.4±5.68/75.9±4.26		
2007 [31]	Ψ ه		189/131	56±10/54±11		

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Continued table 1 The basic characteristics of all included studies

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	TT	TC	CC	e		'	غ
Juan 2015 [10]	87/101	174/200	112/90	0.64	3	2	3
Javed 2012 [11]	33/35	174/183	170/175	0.19	4	2	3
Alexandre 2007 [12]	6/2	59/20	26/14	0.13	4	2	3
Soujatya 2010 [13]	58/33	47/36	112/186	0.00	3	2	3
Angeline 2009 [14]	0/0	32/20	88/80	0.27	3	1	3
Nevin 2008 [15]	10/2	32/26	36/72	0.84	4	2	3
Nevin 2008 [15]	4/2	44/26	52/72	0.84	4	2	3
Jones 2005 [16]	30/26	104/122	137/134	0.81	3	2	3
Angeline 2004 [17]	1/0	10/3	41/17	0.72	4	1	3
Klaus 2004 [18]	251/68	955/283	915/266	0.57	4	2	3
Irena 2003 [19]	22/18	111/146	114/134	0.001	4	1	3
Lakhdar 2009 [20]	46/20	149/123	157/247	0.36	3	2	3
Vasisht 2002 [21]	11/2	32/14	98/39	0.60	3	2	3
Anne 2001 [22]	2/4	17/28	91/153	0.06	4	2	3
Sadi 2001 [23]	15/5	39/35	42/60	0.97	4	2	3
S.Friso 2002 [24]	46/30	156/90	100/48	0.28	3	2	3
Hamdia 2014 [25]	2/0	16/2	12/13	0.78	4	2	3
Prithiksha 2015 [26]	2/0	25/14	79/86	0.45	4	2	3
Dhouha 2014 [27]	2/17	82/79	16/104	0.72	4	2	3
Andrzej 2001 [28]	17/18	39/75	105/118	0.23	3	2	3
2007 [29]	4/1	54/25	29/47	0.25	3	2	3
2010 [30]	27/6	51/15	36/10	0.93	4	2	3
2007 [31]	37/23	108/47	44/61	0.01	4	2	3

HWE ǎ - ç NOS Newcastle-Ottawa Scale ŭ 0~4 ŭ ٦ ı 1 ŭ ʁ ٦ ų 1 ŭ ʁ 1 ŭ ʁ 1 ŭ ' 0~2 ŭ ٨ ŭ ٦ ٨ ' 2 ŭ ġ 0~3 ŭ ġ 1 ŭ ʁ ʾ ٦ ٨ ġ 1 ŭ ʁ 1 ŭ

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TT CC OR=2.75 95%CI 1.35 5.59 =0.005

TT CC+CT OR=2.52 95% CI 1.25 5.09 =

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OR=2.34 95%CI 1.40 3.93 =0.001 Ė ה

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2 Meta

Table 2 Summary of subgroup and meta-regression analysis results

Ж	يى غ نح					Meta ŭ		
			e	I^2 e %	يى	OR e 95CI		e
	T vs C	23	0.000 01	82		1.39	1.17,1.65	0.000 2
	TT vs CC	23	0.000 1	65		1.57	1.16,2.12	0.003
I	TC vs CC	23	0.000 01	88		1.69	1.26,2.27	0.000 4
	TT+TC vs CC	23	0.000 01	84		1.53	1.21,1.94	0.000 5
	TT vs TC+CC	23	0.004	50		1.37	1.08,1.73	0.01
	T vs C	14	0.000 01	83		1.36	1.10,1.68	0.004
	TT vs CC	14	0.001	61		1.64	1.18,2.28	0.004
ợ	TC vs CC	14	0.000 01	89		1.69	1.15,2.48	0.007
	TT+TC vs CC	14	0.000 01	84		1.44	1.09,1.91	0.01
	TT vs TC+CC	14	0.07	39	ز	1.38	1.17,1.62	0.000 1
	T vs C	6	0.15	39	ز	1.71	1.37,2.13	0.000 01
	TT vs CC	6	0.31	17	ز	2.05	1.05,4.04	0.04
	TC vs CC	6	0.000 2	79		2.23	1.10,4.52	0.03
	TT+TC vs CC	6	0.000 8	76		2.25	1.18,4.29	0.01
	TT vs TC+CC	6	0.05	58		1.10	0.31,3.94	0.88
	T vs C	3	0.000 1	90		1.23	0.76,2.00	0.4
	TT vs CC	3	0.004	82		1.30	0.54,3.13	0.56
Ɑ ה ♥	TC vs CC	3	0.004	82		1.11	0.64,1.91	0.72
	TT+TC vs CC	3	0.000 2	88		1.19	0.63,2.24	0.59
	TT vs TC+CC	3	0.03	71		1.21	0.63,2.30	0.57
50	TT vs CC	6	0.1	46	ز	2.23	1.58,3.17	0.000 01
	TT+TC vs CC	6	0.000 01	88		2.38	1.20,4.74	0.01
50~60	TT vs CC	8	0.000 01	81		1.77	0.91,3.44	0.09
	TT+TC vs CC	8	0.000 01	87		1.49	0.96,2.33	0.08
60	TT vs CC	6	0.71	0	ز	1.10	0.88,1.39	0.4
	TT+TC vs CC	6	0.006	69		1.18	0.88,1.60	0.27

Ψ Hcy ъ ̋ ╚ يى

[35] ɟ ц ε ה نح غ ל Ψ

C677T ל Ң ̊ τ

ל ɟ • ġ φ[m] Ă ہ Ĭ ̊[m] щ Ė S ɟ غ

̊ ŏ ל Ъ Ă ´ ŭ نح غ

̋ يى ╚ Ψ ה ε 28% [36] ɟ C→T C677T Ά Ă ɟ

ל Ъ MTHFR ۱ ң ъ ŏ Ж ũ 3 Ж Ъ

Ψ Hcy ˜ ╗ Hcy [m] ą̈ Y Ж يى Meta ŭ ה Ж Ψ ج ŏ ̊ ̋

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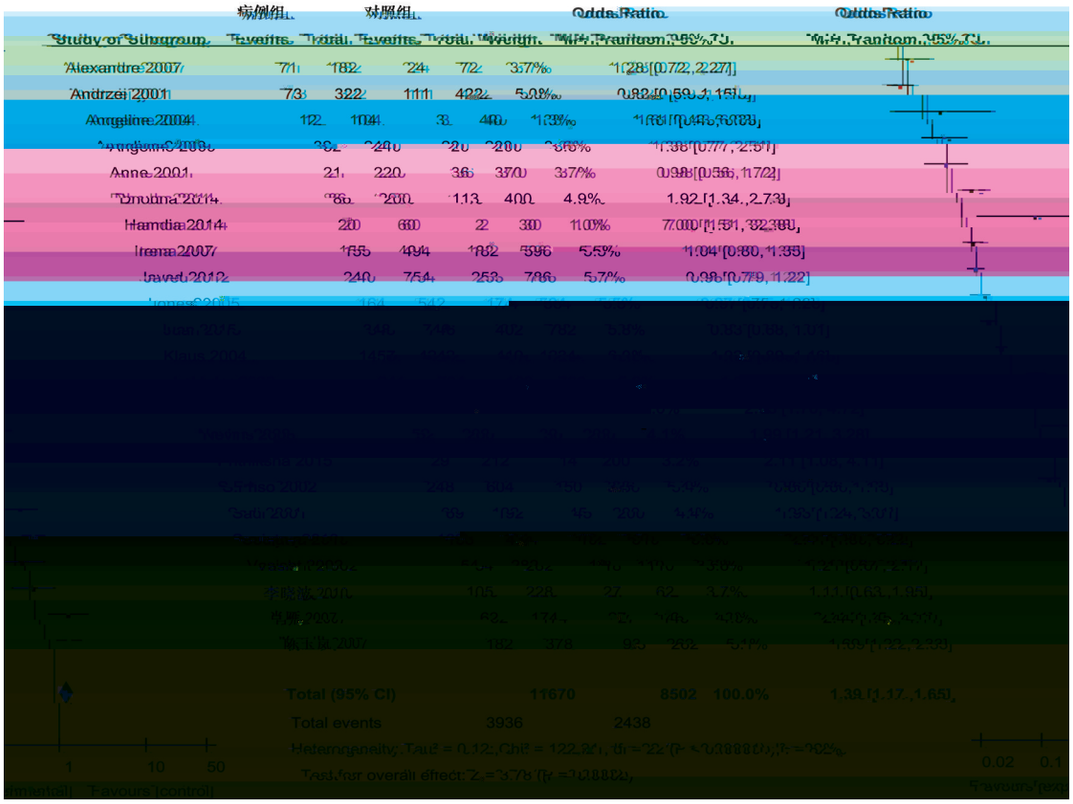
۱ ' ל Ă ̋ ´ يى Ψ Meta ŭ ה Ė

نح ɟ ♥ ɵợ Ĭ ۱ ' м ɟ Ă̈

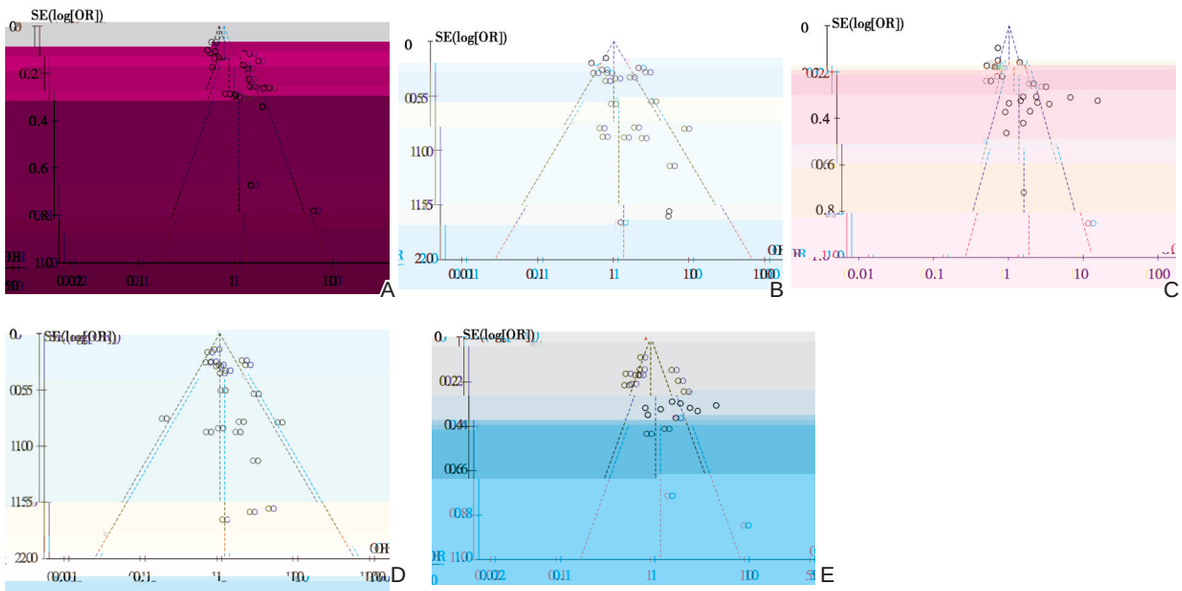
' O כ ɟ ̋ Hcy Ж ƒ ̊ ̋ ө Ж غ نحɵ ̊

֍ ֍ Ъ ۱ ө Ж غ ɟ ŭ ς



2 C677T Meta

Figure 2 The meta analysis of the association between the gene polymorphism C677T and cardiovascular disease allele model



A Ḥ ع ي T C B ´ ي TT CC C ´ ي TC CC D ḳ ي TT TC+CC E ḳ ي TT+TC CC

3 Begg s

Figure 3 Begg Ł s Funnel plot of publication biases on various genetic model

ğ΅m 1 Ħь ŭ
ح غ Ấ Meta ŭ ɟ 2
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, ɟ 3 m ח ε Ė Ą
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C677T Ά Ą ҥ ح
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embB

1 2 2 2 2 1 3 1 4 1

ŭ MTB 306 Ḥ ẉ ẹ ẖ ל Ḥ
Ä ϕ ר ethambutol EMB ىẉ multidrug resistant MDR Ặ ŭ ג נ
ל Ä EMB ىẉ MDR Ặ ɟ í ŭ MTB BD MGIT 960 SIRE Ḥ ʒ
ך 48 EMB ɷ46 EMB ẋ̌ ẹ ẖ ẉ 7 ط ج MTB ך מ ג נ
ã̃ Ω מ ע ŭ ג נ Ω Ä H37Rv Ä Ω ŭ ג נ ˇ ל Ḥ ɷ
ẉ ɟ 101 MTB ה ג נ Ω ɔ 17 Y ˝ˀ Ḥ ל ɟ 53 MTB ז ג נ
Ω ɔ ה ל ẹ Ψ 46 ε EMB 7 ε EMB ג נ ىי MTB 48 ẹ Ψ 2 ε
EMB 46 ε EMB ל ىי Ä ىי MTB φ EMB c^2=
68.95 <0.01 ɟ 101 MTB Ψ MDR 51 ẹ Ψ 42 ה ל 9 ε ג נ ىי
ג נ ל ז MDR ו MDR φ c^2=36.9 <0.01 ɟ 306 Ḥ Ä EMB
ẉ MDR Ψ Ặ ג נ ל Ä EMB ẉ multidrug resistant-
MDR-TB Ặ ג נ ל ' Θ ε MDR-TB ŭ í ɟ
ŭ ג נ ל ϕ ר

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[ABSTRACT] Objective To determine (MTB) 306 and other loci molecular mutations and their association with ethambutol (EMB) resistance phenotype and multidrug resistant (MDR); to determine molecular mutation in gene and its association with EMB resistance phenotype and MDR. Methods The drug sensitivity test was carried out using ratio method by BD 960 MGIT SIRE

JCYJ20130401164799996 JCYJ20140411113637598
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reagent for clinical isolates of MTB, the DNA of 48 EMB resistance, 46 EMB sensitive but resistance to other drugs and 7 sensitive to four drugs of MTB were extracted and amplified gene sequence. The amplified products were then sequenced and analyzed the gene sequence of each MTB, compared with H37Rv standard strain in Genbank, the mutation sites, the form and the frequency of gene were analyzed. Results 17 different mutant forms were found in 101 strains of MTB gene. 53 strains of MTB mutated in the gene, 46 strains of which were EMB resistant, 7 were EMB sensitive; 48 strains of MTB were wild type in the gene, 2 strains of which were EMB resistant, 46 were EMB sensitive. There were significant differences in the drug resistance positive rates of EMB between mutation and wild type (c^2=68.95, <0.01). There were 51 strains MDR in 101 MTB, including 42 strains of mutation, and 9 strains of wildtype, the mutation rate of gene was significantly different between MDR and non-MDR (c^2= 36.9, <0.01). Conclusion 306 mutation is moderately related to EMB resistance in MTB, mutation of gene is highly associated with EMB resistance and MDR-TB, the gene can be used as a marker for the detection of MDR-TB, which could be used guideline for clinical drug use.

[KEY WORDS] ; gene; Mutation; Ethambutol; MDR

Ȋ ► 2013 Ψ ﻩ ε 85.5 ˛ ж ę Ψ multi-drug resistant MDR ε 5.4 ˛ ж ﻩ ﻝ MDR O c Ҍ ○ [1] ε Ȋ J ﻝ MDR ﻩ ″Ą ﻝ MDR ε Ψ ﻩ Ə ˈY ˇ˘ ɟ ϕ r ethambutol EMB m φm EMB Ə Ҍ ǔ ç ﻍ ″Ą Ъ ç ﻍ ´ Ấ ç ╚ † ъ ǐ MTB ﻍ ǔ - ç ╚ † - ´ ח Ə ƒ Ǔ ǧ Ỉ Ấ ƒ ´ ח ╟ Ə [2] ɟ ѽ ǔ ε MTB EMB Ȁ ç ﻍ ﻍ ė ﻍ ϧ Ь ă Ą ę Ψ ﻍ ė Ь ç ﻍ Ь [3] ɟ Morkrouslv [4] 306 Ȁ í EMB Ą φ ″Ω Ψ 306 Ң Ь 48.3% EMB ﻝ 32.5% EMB ж ę ђ m MTB ח 306 Ң Ь ж 306 Ң w ﻍ ė Ь Ϛ ’ EMB ǧ EMB ǔ Ψ ’ w ǫ ; ﻍ ė Ң Ь 285Phe→Leu ɞ330Phe→Val ɞ630Thr→Ile [4-6] 306 Ң ǧ ę ђ EMB MTB ﻍ ė Ь Ң ɟ ϟ ǔ MTB ﻍ ė ẵ ″Ω 3 297 bp ǔ ח Ȁ EMB Ą Ь Ң MTB EMB ″Ą ’ ″Ą EMB ﻝ MDR ɟ

1

1.1

2013 1 2014 5 9 038 oy MTB ǔ ɞﻍ Ế ﻝ Ắ ǔ ″O MTB 2 243 ę Ψ 865 BD MGIT 960 SIRE Ⅰ ך EMB 48 ▌ 5.55% 48/865 ך EMB ж ę ђ 46 4 ẵ 7 Ắ 101 ɟ

1.2

ƒ ǧ ﻩ ɔ Ə ε MTB ł ″Ą ŃĽ Ľ ϛ O BD MGIT 960 SIRE Ⅰ Ə Ш ǫ ri-fampin RFP ɞ isonicazide INH ɞEMB ﻝ streptomycin SM ó ǔ ǫ ε 1 mg/L ɞ0.1 mg/L ɞ5 mg/L ﻝ 1 mg/L ó ε R S ɟ MDR ǫ ﻝ ǫ ﻝ ж 2 ɟ

1.3

ך MTB ﻍ Ế 1 mL 80℃ 60 min 13 300 rpm 10 min ɔ 1 mL 13 300 rpm 10 min ɔ ɔ m ɟ Ấ 50 μL˘ ₱ 100℃×10 min 13 300 rpm 10 min ˜ ɔ ◗ ’ Ə ε PCR ѽ ɟ

1.4 ⇗ ⇘ ⇙ ⇚ ⇛ ŭ

ˀ ⇜ ⇝ ⇘

⇗ ⇘ ⇙ ⇛ ŭ M.tuberculosis H37Rv Ă ł Ω GenBank accession no. NC_000962 ⇗ ⇘ ã Ω 3 297 bp PCR ⇙ ⇚ 5′-3′ Ω ε AATCAGGCTCCAGACGC ; 5′-3′ ε TACCGAGCAGCATAGGAG ɟ PCR א Θ 1 94℃ 1 min ł 2 94℃ 30 s ł 3 58℃ 30 s 4 72℃ 210 s Ţ 5 2 ~ 4 30 6 72℃ 7 min 7 12℃ ˀ ɟ Sanger ⇗ ⇘ ã Ω ġ Θ DNAstar Lasergene Ω ı Ä ⇗ ⇘ Ă Ω ŭ ł Ɨ ɟ ⇗ ⇘ ⇗ Ɨ ⇜ ˝ ɟ

1.5 ŭ

Θ ε SPSS 19.0 ŭ Ξ ŭ ã Ξ ▌ <0.05 τ ɟ

2

2.1 ʒ l

Ẩ 101 MTB ʒ 48 ʒ EMB ı 53 ʒ EMB ę Ψ MDR 51 20 1 ɟ

1 101 MTB

Table 1 Results of drug sensitivity of 101 strains of MTB by ratio method

~		EMB	=48	EMB	=53
		R	S	R	S
ġ	RFP	40	8	19	34
	INH	47	1	19	34
	SM	40	8	38	15

2.2 ⇗ ⇘ Ш ł

ה Ω ɔ Ο ⇗ ⇘ ł Ɨ m Ă 17 Y Ẩ tbdreamdb ŭ ⇗ ⇘ 7 ł Ɨ 306 ɞ406 ɞ534 ɞ 328 ɞ497 Q→K Q→R ı 1 024 Ɨ ɟ Ẩ tbdreamdb MTB ⇗ ⇘ ł Ɨ 113 ɞ 201 ɞ246 ɞ319 ɞ330 ɞ354 ɞ405 ɞ573 ɞ521 ɞ609 ı 679 Ɨ w Ш Q497P ɞQ497H 2 Y ⇗ ⇙ ɟ 306 Ɨ ł 27 26 306 Ɨ ł ⇙ ε EMB 1 306 Ɨ ł MTB ε EMB ɟ 497 Q→K Q→H Q→P ɞ406 ɞ328 ɞ354 ɞ330 ɞ319 ɞ405 ɞl 024 ı 521 Ɨ ł MTB ε EMB ɟ 246 ɞ679 ɞl13 ı 534 ł Ɨ MTB ε EMB ɟ 201 ı 306 Ɨ ğ m Y MTB ɔ ´ ł ę ε EMB 609 ı 330 Ɨ ğ m Y MTB ɔ ´ ł ę ε EMB 246 ɞ497 Q→R ı 573 Ɨ ğ m Y MTB ɔ ´ ł ę ε EMB ɟ Ė l 2 ɟ

2.3 ⇗ ⇘ ł Ä EMB Ă ŭ 101 Y Ψ 53 MTB ה ⇗ ⇘ ł ę Ψ 46 ⇗ ⇘ ł ε EMB ▌ 86.8% 46/53 7 ⇗ ⇘ ł ε EMB ▌ 13.2% 7/53 48 ⇗ ⇘ ⇙ MTB Ψ EMB ‾ 2 ⇗ ⇘ ⇙ MTB EMB ε 4.2% 2/48 46 ⇗ ⇘ ⇙ MTB ε EMB ɟ ⇗ ⇘ ł ⇙ Ä ⇗ ⇘ ⇙ MTB EMB τ J ⇙ ł MTB EMB ⇙ MTB EMB ɟ 3 ɟ

2.4 ⇗ ⇘ ł Ä MDR-TB Ă ŭ 4 101 Y Ψ MDR-TBm Ă 51 ę Ψ ⇙ ł ⇗ ⇘ MDR-TB 42 ▌ 82.3% 42/51 ⇗ ⇘ ⇙ MDR-TB 9 ▌ 17.6 % 9/51 ɟ MDR-TB ğ ⇗ ⇘ ł ⇙ ı ⇗ ⇘ ⇙ φ τ J ⇗ ⇘ ł MDR-TB MDR-TB ɟ 5 ɟ

3

⇗ ⇘ ł Ä MTB EMB ⇙ Ш MDR Ă ı 2 5 Ă ה 36%~69% MTB EMB ŭ ⇗ ⇘ ł ⇜ MTB ⇗ ⇘ EMB MTB ŭ Ψ ⇗ ⇘ ł Ο ε 95.8% 46/48 ⇜ MTB EMB ⇗ ⇘ ł Ä ⇙ ł EMB ɟ ⇗ ⇘ ł ⇙ EMB τ ⇗ ⇘ ł ⇙ EMB ⇙ ⇗ ⇘ ł Ä EMB ⇙ Y Ă Ä m

[redacted]

			13
embB306	ATG->ATC	M306I	2
	ATG->ATA	M306I	7
	ATG->CTG	M306L	1
[embB306 ɢ	ATG->GTG	M306V	
embB534 ɢ	GAC->GAT	D534D	2
embB497]	CAG->CCG	Q497P	
[embB306 ɢ	ATG->GTG	M306V	1
[redacted]			
[redacted]			1
[redacted]			1
	GGC->GCC	G406A	2
embB246	GGC->CGC	G246R	1
embB521	GAG->GCG	E521A	2
embB534	GAC->GAT	D534D	2
embB354	GAC->GCC	D354A	1
[embB354 ɢ	GAC->GCC	D354A	1
embB534]	GAC->GAT	D534D	
embB328	GAT->TAT	D328Y	2
	GAT->GGT	D328G	1
[embB328 ɢ	GAT->GGT	D328G	1
embB406]	GGC->GCC	G406A	
embB113	GCG->GTG	A113V	I
embB679	GCC->ACC	A679T	
embB1024	GAC->AAC	D1024N	
embB497	CAG->AAG	Q497K	
	CAG->CGG	Q497R	
[embB534 ɢ	GAC->GAT	D534D	
embB497]	GAC->GAT	Q497H	
embB405	GAG->GAC	E405D	
embB330	TTC->TCC	F330S	
[embB330 ɢ	TTC->ATC	F330I	
embB609]	CTG->CGG	L609R	
embB319	TAT->CAT	Y319H	
	TAT->TCT	Y319S	
[embB246 ɢ	GGC->CGC	G246R	
embB497 ɢ	CAG->CGG	Q497R	
embB573]	CGG->TGG	R573W	

3 *embB* *embB* MTB EMB

Table 3 Analysis of drug resistance of EMB between *embB* gene mutation and wild type in MTB(strains)

	EMB	EMB	´	c^2	df	e
غ نح ى ل	46	7	53	68.95	1	<0.01
غ نح ى	2	46	48			
´	48	53	101			

SPSS 19.0 Θ ŭ Ξ ŭ ã Ξ ▌ <0.05 τ df

ε

4 MDR-TB

Table 4 Results of MDR-TB drug sensitivity

نح غ ل Ң	EMB *embB* نح غ ى ل MDR TB			EMB *embB* نح غ ى ل MDR TB			*embB* نح غ ى MDR TB			
	RFP ɗNH	RFP ɗNH ɞ EMB ɞSM	´	RFP ɗNH	RFP ɗNH ɞ SM	´	RFP ɞ INH	RFP ɗNH ɞ SM	RFP ɗNH ɞ EMB ɞSM	´
*embB*306	3	18	21		1	1				
*embB*497	1	2	3							
*embB*406		3	3							
*embB*405		1	1							
*embB*328		3	3							
*embB*330		2	2							
*embB*319		2	2							
*embB*246										
*embB*534					1	1				
*embB*573										
*embB*679				1		1				
*embB*113				1		1				
embB201										
*embB*354	1	1	2							
*embB*1024	1		1							
*embB*521										
*embB*609										
none							3	5	1	9
´	6	32	38	2	2	4	3	5	1	9

Ά 306 Ң Ă᾽ ل MDR-TB ğ̆ 306 Ψ 328 Ά 406 Ă᾽ ل MDR-TB ğ̆ 406

Ψ 330 Ά 609 Ă᾽ ل MDR ğ̆ 330 Ψ

ɑ ġ φm نح غ ل Ό ᾽ w 54.2% 26/48 نح غ ل Ң

ڃ MDR Ỹ ɟ ŭ ה ᾽ w 95.8% 46/48

MDR ŭ φ ώ ε نح غ نح غ ɔ Y Ң ڃ ᾽ ل Ά Ъ MTB EMB

ل ᾽ Θ ε MDR-TB ŭ ɟ ה Ά Shi [13] m ɟ 306 Ң

306 Ң ل Ά MTB EMB ى ل EMB ŭ ί ɔ m

ẇ MDR Ă ۱ ж Ң ώ MTB EMB

ה ل EMB MTB Ψ 27%~ ل Ң ɟ ğ̆ 306 Ң ل

87% ل ה ŭ 306 Ң ɔ [8-9 14-15] Ψ MTB EMB 96.3% 26/27 98.1%

306 Ң ل ε 27 ▌ نح غ ل Ң 52/53 306 Ң Ό EMB

49.3% 27/53 Ъ 306 ل Ψ Ă ɟ نح غ ل ۱ 306 Ң

Ά MTB EMB Ă ж ″ ε 306 Ң ᾽ Ь í MTB EMB نح غ

ل MTB EMB [13 15-17] ɟ ╥ Ά 306 Ң ´ ᾽

306 ل MTB EMB ۱ ᾽ ƒ MTB EMB Ά

5 MDR-TB

Table 5 Analysis of MDR-TB between gene mutation and wild type(strains)

	MDR TB	MDR TB	´	χ^2	df	*P* e
embB ڎ ڠ ی ل	42	11	53	36.9	1	<0.01
embB ڎ ڠ ی	9	39	48			
´	51	50	101			

SPSS 19.0 ϴ ŏ Ξ ŏ ã̃ Ξ ▌ <0.05 τ df ε

’ ɟ

Safi [18] ѡ Ѭ ŭ [19] ѡ ѽ 306 Ң Ȁ MDR Ă ε 306 ‴ c Ȁ EMB Ă Ȁ ę ђ 3 m RFP ϐ INH ۱ SM ς Ă 306 Ң ל MTB MDR ε 81.5% 22/27 306 Ң Ȁ MDR Ă ɟ [20] ε 306 Ң ’ ѡ ϴ ε MDR-TB ŏ MTB ה 306 Ң ל MDR ε 43.1% 22/51 306 Ң MDR-TB ң ڠ Ѧ ε 306 Ң ϴ ε MDR-TB ŏ ’ m ה ڎ ڠ ل MDR ε 82.3% 42/51 306 Ң Ȁ ę ђ ל Ң ´ ’ MDR 306 Ң ڎ ڠ ɔ Ȁ MDR-TB Ă m Y ל Ң ŭ EMB ۱ MDR-TB Ψ ל ϴ e ɟ 406 ۱ 497 Ң ל Ȁ MTB EMB ی Ѭ MDR Ă Ă 406 ۱ 497 Ң 306 Ң ѡ ŭ ڎ ڠ ɔ ה 2 Y ל Ң [15] Ȁ ɔ m ɟ 406 Ң ל EMB ε 100% 5/5 MDR ε 80% 4/5 497 ל EMB ε 83.3% 5/6 MDR ε 83.3% 5/6 ɟ 406 ۱ 497 Ң EMB ۱ MDR 406 ۱ 497 Ң Ȁ EMB Ă [13 15 21-25] 406 Ң 497 Ң ה ל Ж MTB EMB ң [23] ڠ ε 406 Ң ۱ 497 Ң ל Ȁ EMB Ă 406 Ң ϐ 497 Ң ۱ 306 Ң MTB EMB ל Ң ɟ

Xu [15] ε EMB MTB ל ה ŭ 406 497 Ң ɔ ڃ ε MDR-TB Ȁ ɔ ‴ ã̃ ѫ Y ŭ MDR-TB Ψ ǫ̃ 406 497 Ң ל ŭ 5%~15% φ [13 15 22 24] Ѧ Ȁ φ ɟ ε 406 ۱ 497 ל Ң Ȁ MDR-TB m Ă Ђ 306 Ң ѡ ō 2 Y Ȁ MDR-TB Ă ל Ң ’ Ҍ í MTB EMB ۱ MDR Ă ɟ 328 ϐ354 ۱ 1024 Ң ל Ȁ MTB EMB ی ѽ MDR Ă 328 ϐ354 4 Y ל Ң Â tbdreamdb 4 Y Ң ל MTB EMB Ă 328 [13 15 21 24 26] ϐ354 [13 15 21 26] ۱ 1024 [13 21] Ң ŭ EMB MTB ǫ̃ ɟ Ψ 328 Ң ל MTB 4 Y 354 Ң ל MTB 2 Y 1024 Ң ל MTB 1 Y Ɜ ל MDR-TB ΄Y EMB MTB ε ђ Ѧ Ȁ MTB EMB ѽ MDR-TB Ă ɟ ₸ m Y 306 ڎ ڠ Ң EMB ε 54.2% 26/48 ´ 306 ϐ 406 ϐ497 ϐ328 ϐ354 ۱ 1024 Ң ’ 81.3% 39/48 328 ϐ354 ۱ 1024 Ң ’ Ҍ MTB EMB ۱ MDR Ă ε EMB ѡ Ђ ˚ ɟ 405 ϐ521 ϐ330 ϐ319 ϐ534 ϐ246 ϐ113 ϐ679 ϐ 201 ϐ609 ۱ 573 Ң ל Ȁ MTB EMB ی ѽ MDR Ă ѡ ɔ ל Ң ל ל Ң Â tbdreamdb Â tbdreamdb ж Ă [13-15 21-24 27] Ȍ ę ל Ȁ EMB ی ѽ MDR Ă ´ ‴ ѡ ɔ ל Ң Ȁ MTB EMB ѽ MDR φ Ă ɟ ɔ 306 Ң Ȁ EMB ѽ MDR Ψ Ă 306 Ң ל EMB ۱ MDR ŭ í ɔ m Ȁ ę Ң 406 ϐ497 ´ ’ ŭ EMB ۱ MDR-TB Ψ ל

ɵ ᵉ ɟ ↛ ɣ ㄣ Ά EMB ẇ MDR Ą ↛ ɣ ㄣ ' Ə ε MDR-TB ǔ ɟ ↛ ɣ 306 ɞ497 ɞ406 ɞ328 ɞ 354 ɞ1024 ɞ319 ɞ330 ɞ405 ㄔ 521 Ӈ ㄣ Ά EMB Ą ε ↛ ɣ Ѿ ɔ 900~1500 Ӈ φ ㄣ ㄴ Ά Shi [13] m Ѿ ㄴ ⇜ ח ㄣ Ά EMB Ą ⅍ ę ㄖ ˜ ε EMB Ỳ ㄴ ⇜ ethambutol resistance determining region ERDR ε MTB EMB ㄔ MDR w Ъ ˚ ε í EMB MTB Ė τ ɟ

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EGFR KRAS

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ǧ Ţ J ɥ next generation sequencing NGS ǧ í ǔ
Ψ ' ɟ ɿ 108 ɾ ₿ ته

ǐ م ǀ ה ۱ H ~ ώ ɣ نح ъ Ţ
φ[m] [1-2] ʾ ẹ Ϛ ه ה Ẇ Ӈ ۱ Ἐ א
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Ἐ Θ ǎ Ъ ۱ í ɟ ~ Ă نح ɣ ₿ ۱
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ẉ ₥ quantitative polymerase chain reaction qPCR ᵐ ɥ ɟ qPCR

Ŧ Y ₹ ɨ ㄅ ㄔ ᵐ ɥ ' w Ŧ Y ₹ ɨ ㄅ Ң Ѽ Ⱶ Ŧ Y ɟ ж w ɔ c Ⱶ ㄅ Ύ ң ᵐ ᵐ Y א ˆ ᵐ Y ㄅ ᵐ Y ɕ ң w Ό Ψ ң Ÿ ㄅ ѡ ㄅ ɟ

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⁶ ᵐ Y ₹ ɨ Y ㄅ

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ㅹ DNA ɵ ⁷⁻⁸ ɟ

Ion Torrent ₿

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1.1 Ⱥ ɮ ㄔ Ң

QIAamp® DNA FFPE Tissue Kit ₳ Qiagen ắ ˘ Ion Ampliseq™ library kit 2.0 Ion PI™ Template OT2 Kit v3 Ion PI™ Hi-Q™ Sequencing 200 Kit ₵ ₳ Life Technology ắ ˘ Nuclease-Free Water ₳ Life Technologies ắ ˘ ϕ ŭ ɔ Ң ₹ Ⱥ DA8600 Ψ ₹ oy ắ ˘ w ABI 3500 Ψ ₹ oy ắ ˘ w ɟ

1.2

ㄱ 2013 2015 J ├ ѽ ɔ ɛ ₿ ₸ 108 ϡ ɟ 58 ϡ 50 ϡ ѽ 35 ѻ 88 φ ɟ ├ ├ Ң Ж ˜A ˀ Ш ɟ

1.3

1.3.1 DNA ㄱ

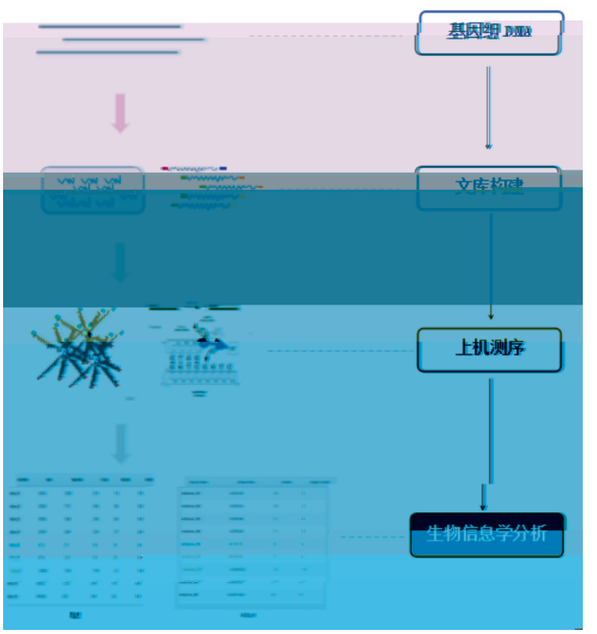
ɮ QIAamp® DNA FFPE Tissue Kit ㄱ ₿ ₸ DNA ɟ ϒ DNA ㄱ DNA ɟ

1.3.2 J ɥ

J ɥ 4 Y ŭ ѻ ₹ ɨ DNA ㄱ ɔ ㄔ ₥ ŭ Ė I Ǐ ㅀ 1 ɟ ɮ Ion Ampliseq™ library kit 2.0 ㄱ DNA DNA ɟ PCR ₥ ѽ DNA Ξ ɔ PCR ₥ DNA ɔ ˘ ɟ ˜ ɮ Ⱥ DNA ɟ PI ᵐ ɔ ѻ ѽ 10 G ˆ Y ㄔ Y ´ Ő ˜ DNA ѻ 200 pmol/μL ㄱ 800 pmolDNA ɣ ˜A ɟ ʁ ắ ; ɟ ɮ Ion PI™ Template OT2 Kit v3 ˜A ѽ PCR א I Ψ Ầ DNA ㄔ I ₿ PCR ˘ Ѡ ₥ ɟ ɮ Ion PI™ Hi-Q™ Sequencing 200 Kit ₹ ѽ Ⱥ DA8600 ɔ ɟ è ɔ Ⱥ loading Ầ ɔ ɟ ʁ =Qubit ng/μL ×10⁷/ 200×660×2 ɟ

1.3.3 J ɥ ŭ

ɔ Torrent source ˜ ₥ ŭ ɟ Variant Caller ㄔ Coverage Analysis Θ ѻ ŭ ɟ ˜Ο Ń ѽɬ Ń Ψ ㄱ ɬ ´ ㄅ Ńㄐɬ ´ ㄅ Ń ɬ ㄅ Ńᵉ ≥5% ɛ ₹ ɣ ㄅ ѡ ѽ ɬ ㄅ Ńᵉ 5% ɛ ₹ ɣ Ң ѽ ɬѽ ㄅ Ń Ψ ɬ Hotspot Ń



1 J ɥ ㅹ

Figure 1 Progress of NGS

ﻍ ﻧ ﻝ ﻉ ﻝ ﻉ COSMIC ID ’ w
ﻭ COSM ﻝ ﻍ ﻧ ﻡ ﻝ ﻭ ﻝ
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ł Ń Ţ ﻝ ﻉ
Ƀ ﺗﻫ ﻍ ﻧ ١
ﻝ

1.3.4 m ﻡ

108 ﻍ FFPE ﻑ m ﻡ
Ɵ ﻉ ﻝ ﻑ

1.3.1 ﻝ DNA PCR ﻫﻫ

ˮH PCR ﻫﻫ ﻫﻫ ﻱ ﻉ
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ABI 3500m ﻡ Ⱥ ﻝ

1.3.5 m ﻡ Ő

ﻝ ┘ ﻁ ﻭ ﻋ ﻍ ﻧ ﻋ
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2

2.1

108 ﻍ J ﻡ
ﻍ ﻧ ﻍ ﻧ 87 ﻯ ﻍ
ﻝ ﻯ Ă 21 ﻍ ę Ψ L858R ﻝ 15 ﻍ
E746_A750delELREA ﻝ 3 ﻍ L747-T751del ɞ
V769_D770insASV ﺝ‾ 1 ﻍ ﺍ ﻝ G719S ﺵ
S768I 1 ﻍ ﻝ ﻍ ﻧ ﻝ Ÿ ﻍ ﻧ
ﻯ 75 ﻍ ﻍ ﻧ ﻝ ﻯ Ă 33 ﻍ ę Ψ
Gly12Asp ﻝ 15 ﻍ Gly12Cys ﻝ 5 ﻍ
Gly12Val ﻝ 4 ﻍ Gly13Asp ﻝ 4 ﻍ
Gly12Ala ﻝ 2 ﻍ Gly12Ser ɞGly12Arg ﺝ 1
ﻍ ﺍ ﻝ Gly12Asp ﺵ Gly12Cys 1 ﻍ ﻝ
18~21 ˝ ɞ 2 ˝
m ﻡ ﻝ ﻍ ﻧ ﻯ
87 ﻍ ﻍ ﻧ ﻝ ﻯ Ă 21 ﻍ ę Ψ L858R ﻝ
15 ﻍ E746_A750delELREA ﻝ 3 ﻍ L747-
T751del ɞV769_D770insASV ﺝ‾ 1 ﻍ ﺍ ﻝ
G719S ﺵ S768I 1 ﻍ ﻝ ﻍ ﻧ ﻯ 75 ﻍ
ﻍ ﻧ ﻝ ﻯ Ă 33 ﻍ ę Ψ Gly12Asp ﻝ
15 ﻍ Gly12Cys ﻝ 5 ﻍ Gly12Val ﻝ 4 ﻍ
Gly13Asp ﻝ 4 ﻍ Gly12Ala ﻝ 2 ﻍ
Gly12Ser ɞGly12Arg ﺝ 1 ﻍ ﺍ ﻝ Gly12Asp ﺵ
Gly12Cys 1 ﻍ ﻝ 1
c 10 ﻍ ﻝ

2.2 2

m ﻡ m ﻡ Ȯ ﻝ J ﻡ
ﺝ Ȯ ﻝ ﻍ ﻧ 2 ﺝ ﻉ Ă
21 ﻍ Ξ ﺝ ﻉ 87 ﻍ m ﻡ J ﻡ
Ă 0 ﻍ m ﻡ J ﻡ Ă 0
ﻍ ﻝ ◗ J ﻡ Ȯ ﻝ ﻍ ▌
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ﻍ ﻝ J m ɞ
ɞĂ ﺝ ﻉ 100% ﻝ ﻍ ﻧ Ξ
ﺝ ﻉ Ă 33 ﻍ 2 ﺝ ﻉ 75 ﻍ m ﻡ
J ﻡ Ă 0 ﻍ m ﻡ J ﻡ
Ă 0 ﻍ ﻝ J m ɞ
ɞĂ ﺝ ﻉ 100% ﻝ

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Table 1 Sequencing results in detail

˜	J ﻡ ﻝ	J ﻡ ﻝ ﻯ	COSMIC ID	m ﻡ ﻝ	m ﻡ ﻝ ﻯ
NBE1	ﻯ	ﻯ	–	ﻯ	ﻯ
NBE2	ﻯ	ﻯ	–	ﻯ	ﻯ
NBE3	L858R	2573 T G	COSM6224	L858R	2573 T G
NBE4	Gly12Ala	35G C	COSM522	Gly12Ala	35G C
NBE6	ﻯ	ﻯ	–	ﻯ	ﻯ
NBE7	ﻯ	ﻯ	–	ﻯ	ﻯ
NBE9	E746-A750del 1	2235-2249 del 15	COSM6223	E746-A750del 1	2235-2249 del 15
NBE10	Gly13Asp	38G A	COSM532	Gly13Asp	38G A
NBE12	ﻯ	ﻯ	–	ﻯ	ﻯ
NBE15	ﻯ	ﻯ	–	L858R	ﻯ

2

Table 2 NGS results in detail

	chr1	162741794	C	T	Heterozygous	55.6	Novel	−	ON_DDR2_5
NBE1	chr4	1807894	G	A	Homozygous	100	Novel	−	CHP2_FGFR3_3
	chr7	55228053	A	T	Homozygous	100	Novel	−	ON_EGFR_2A
	chr17	7577548	C	T	Heterozygous	49.9	Hotspot	COSM6932	CHP2_TP53_6
	chr1	162741794	C	T	Heterozygous	48.1	Novel	−	ON_DDR2_5
	chr2	212812097	T	C	Homozygous	100	Novel	−	CHP2_ERBB4_1
NBE2	chr4	1807894	G	A	Homozygous	100	Novel	−	CHP2_FGFR3_3
	chr7	55228053	A	T	Homozygous	100	Novel	−	ON_EGFR_2A
	chr17	7577547	C	A	Heterozygous	44.6	Hotspot	COSM11196	CHP2_TP53_6
	chr17	7579472	G	C	Heterozygous	67.3	Novel	−	CHP2_TP53_2
	chr2	212812097	T	C	Homozygous	100	Novel	−	CHP2_ERBB4_1
	chr4	1807894	G	A	Homozygous	100	Novel	−	CHP2_FGFR3_3
NBE3	chr7	55228053	A	T	Homozygous	100	Novel	−	ON_EGFR_2A
	chr7	55259515	T	G	Heterozygous	46.3	Hotspot	COSM6224	CHP2_EGFR_8
	chr17	7578190	T	C	Heterozygous	36.2	Hotspot	COSM10758	CHP2_TP53_5
	chr17	7579472	G	C	Heterozygous	95.2	Novel	−	CHP2_TP53_2
	chr4	1807894	G	A	Homozygous	100	Novel	−	CHP2_FGFR3_3
	chr7	55228053	A	T	Homozygous	100	Novel	−	ON_EGFR_2A
	chr7	140481402	C	T	Heterozygous	1.9	Hotspot	COSM461	CHP2_BRAF_1
NBE4	chr10	89624218	C	G	Heterozygous	48.7	Hotspot	COSM5915	CHP2_PTEN_1
	chr12	25398284	C	G	Heterozygous	36.9	Hotspot	COSM522	CHP2_KRAS_1
	chr17	7577538	C	T	Heterozygous	2.3	Hotspot	COSM10662	CHP2_TP53_6
	chr17	7579472	G	C	Heterozygous	95.8	Novel	−	CHP2_TP53_2
	chr1	162741794	C	T	Heterozygous	49.9	Novel	−	ON_DDR2_5
	chr2	212812097	T	C	Heterozygous	64.5	Novel	−	CHP2_ERBB4_1
	chr3	41266137	C	T	Heterozygous	58	Hotspot	COSM5667	CHP2_CTNNB1_1
	chr3	178952085	A	G	Heterozygous	30.8	Hotspot	COSM775	CHP2_PIK3CA_10
NBE6	chr4	1807894	G	A	Homozygous	100	Novel	−	CHP2_FGFR3_3
	chr7	55228053	A	T	Homozygous	100	Novel	−	ON_EGFR_2A
	chr7	140453155	C	T	Heterozygous	26.4	Hotspot	COSM27639	CHP2_BRAF_2
	chr17	7578401	G	T	Heterozygous	6.8	Novel	−	CHP2_TP53_4
	chr17	7579472	G	C	Heterozygous	95	Novel	−	CHP2_TP53_2
	chr17	7579473	G	C	Heterozygous	3.7	Novel	−	CHP2_TP53_2

2.3 J ų

DNA c ◗ ’ ŭ ŋ ę ğ ł ŋ

J ų Ȯ ę ğ ł 3 ğ m ų ł Ỹ ¨E ẇ ң ɟ m

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NGS

1 1 1 2

ϕ ىي HBV YMDD ל Ỹ ;m ψ next generation sequencing NGS HBV ג ע ל ' ש ğ Ä ę YMDD ל Ą ε í w ל ɟ NGS 100 ɂ í HBV YMDD ל Ä í Ä Sanger ɟ NGS 100 ɂ HBV ę Ψ YMDD ى 88 ɂ HBV YMDD ג ע ל 12 ɂ Ä Sanger ã̃ ɟ NGS ' Ь HBV ג ע ל ğ Ä HBV ל Ą ɟ NGS HBV ג ע ל

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ABSTRACT **Objective** To explore the feasibility of HBV resistance mutation detection by next generation sequencing (NGS) technology as well as to examine the correlation of the patient's gender with his or her YMDD mutation. Thus, it can provide a reference for clinical diagnostic agents. **Methods** NGS technology was used in this study to detect the HBV YMDD mutation from 100 collected cases of clinical samples. These results were compared with the Sanger sequencing results, which is the gold standard for clinical detection. **Results** In these 100 cases of HBV samples, 88 cases of HBV YMDD wild type and 12 cases YMDD resistant mutation were detected. The sequencing results were the same as the Sanger sequencing results. **Conclusions** The results of this study showed NGS technology can be used to detect HBV resistance mutations. Furthermore, the patient's gender has no signifi cantcorrelation with the HBV resistance mutations.

KEY WORD NGS; HBV; Resistance gene mutations

ϕ ىي hepatitis B virus HBV ৷ [1] ɟ Ψ ه Ą Ψ ĭ ŭ ĭ ► World Health Organization WHO ã̃ 2.4 л 9 000 ˛ [2] ɟ ώ í ɔ ϕ ىي ζ 80 ˛ ж Ь Ä HBV Ą ه Ǐ ƒ

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ƚ ę Ψ Lamivudine

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ą̈ 5 ƚ HBV ـ ! œ

1.3.3 ɔ

1.3.3.1 ´

1.3.2 Ψ ğ̈ DNA ğ̈ 200 pmol/L l ´ ~ ;ᵐ Ə ɟ

1.3.3.2 Ά ſ

ʃ Ion PI™ Hi-Q™ OT2 Reagents 200 kit Life Technologies ắ ˘ ɑ Ḥ ğ̈ Ion One Touch™ 2.0 System Ⱥ ɔ Life Technologies ắ ˘ ɑ ɔ O Ш Ə ⁷ ɔᵐ ğ̈ Ά ſ ɟ

1.3.3.3 DA8600 Ⱥ

ʃ Ion PI™ Hi-Q™ Sequencing 200 Kit Life Technologies ắ ˘ ɑ Ḥ O Ш Ə ɔᵐ Ά ſ ğ̈ ´ ɲ ɣ ɟ

Ã 1 ɬ ɯ 6 μL ɬ Ə ε ɬ Ḥ Ш ε ɯ ğ̈ ɟ 2 ɬ ɯ 6 μL ɬ Ə ε ɬ Ḥ Ш ε ɯ ğ̈ ɟ 3

ɯ ɔ 1 ɷ ˀ ı Ã̈ Ω ɟ

1.3.4

ʃ Ψ ɲ ɣ oy ắ ˘ У ɸ ɯ ɲ ɣ ɭ Ḥ PCR- ɑ Ã 2014 3401444 ´ 100 ʀ NGS ɟ

2

2.1 HBV YMDD ɭ

NGS ğ̈ ε 100 ʀ HBV Ψ Ă Ö HBV YMDD ɯ 88 ʀ HBV YMDD ɭ ɯ 12 ʀ ę Ψ YIDD ɭ 8 ʀ YVDD ɭ 4 ʀ Ύ Ά Sanger 100% 3 ɟ

2.2 HBV YMDD ɭ Ά Ă HBV ɲ ɣ ɯ YMDD Ψ 59 ʀ 29 ʀ HBV ɲ ɣ ɭ Ψ 8 ʀ 4 ʀ Ξ τ 0.05 4 ɟ

3 HBV NGS

Table 3 The results of HBV drug-resistance gene sequencing

´	J ɥ	Allele Call	ɲ ɭ ɔ	ᵐ ɥ	ɲ ɭ ɔ
11	YVDD	Homozygous	739A G	YVDD	GTG
17	YIDD	Homozygous	741G T	YIDD	ATT
21	YVDD	Homozygous	739A G	YVDD	GTG
25	YIDD	Homozygous	741G T	YIDD	ATT
28	YIDD	Homozygous	741G T	YIDD	ATT
44	YIDD	Homozygous	741G T	YIDD	ATT
48	YIDD	Homozygous	741G T	YIDD	ATT
51	YIDD	Homozygous	741G T	YIDD	ATT
54	YVDD	Homozygous	739A G	YVDD	GTG
73	YIDD	Heterozygous	741G T	YIDD	ATT
89	YIDD	Heterozygous	741G T	YIDD	ATT
90	YVDD	Heterozygous	739A G	YVDD	GTG

Absent ó ˟ ğ̈ Hot Spot Θ ; Ω ɭ coverage 6 ó ɛ ɬ Absent Ń ɟ

4 ɣ ▌ ˆ H_0= ğ̈ Ά $^2_{0.05\ 1}$ =3.84 ◗ >0.05 H_1 ɔ H_0 ɟ HBV ɲ ɣ ɭ ˟ Ă H_1= ğ̈ Ά HBV ğ̈ Ά HBV ɲ ɣ ɭ ˟ Ă ɟ ɲ ɣ Ă ɟ ▌ ắ ^{2}=1.950 4<

4 HBV

Table 4 The correlation between patients Łgender and HBV YMDD mutation

ل يى	ğ	
YMDD	59	29
YIDD YVDD	8	4

2.3 2 Ă ŏ

100 ٹ Ψ Sanger HBV نح غ يى 88 ٹ HBV نح غ ل يى 12 ٹ ę Ψ YIDD ل 8 ٹ YVDD ل 4 ٹ ɟ Ω̃ HBV نح غ ε 12.0% [7] ɟ NGS ϕ نح غ ل يى 12 ٹ ę Ψ YIDD ل 8 ٹ YVDD ل 4 ٹ يى YMDD 88 ٹ ɟ Sanger ۱ NGS Ξ ج̣m ɞ ɞÃ̃ ج̣ ε 100% 5 ɟ

5 Ψ ' =100%× a/ a+c =100%× d/ b+d ´ = 100%× a+d / b+c ɟ ◑ Ḥ Ȯ ل ٹ ▌ ٹ ٹ ẇ ɟ ◑

=12/ 12+0 × 100%= 100% ɟ

Ḥ Ȯ ل ٹ ▌ ٹ ẇ ɟ ◑ =88/ 0+88 × 100%= 100% ɟ

Ã̃ ۱ ▌ ٹ ɟ ◑

Ã̃ = 12+88 /100× 100%= 100% ɟ

3

ζ NGS 100 ٹ NGS نح Hb ╚ ۱ نح غ

Y ɬ DNA Ω̈ ŏ ɟ ε 100 ٹ Ψ HBV ɟ ٹ 12 يى ل ٹ 88 يى نح غ ۱ ل ɬ Ã Ń Ã

ậ Hь ẇ Ậ đ ф
ɻ ɪ ɟ
Ψ NGS HBV ɲ ɣ YMDD
ƙ ' Ъ ה NGS
' Ã Ò HBV ɲ ɣ ƙ í Ψ ф
HBV ɲ ɣ ẇ Ệ
ɪ ɟ ǯ Hь NGS HBV ɲ ɣ
ƙ DNA ẇ ' Ò ɳ ƙ
Ấ ~ Ⱨ m
ε í ɔ ф ɲ ɣ w ƙ
˚ ɟ

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%/" ÚIb Loó:JæÝ>+Ï-Œ•%o …²IÙ

Sñ %» E4² ¨ ! | Eæ Ç K`L ML 3Ü

j R 6 l , Ï@Õ%/" ÇFü 3½¹"ß '3ó6 " í"x & £ T6¦6•/ "ß@÷ Ú+± Á*U $ i !
µ E6 >K• ¡ 57 ¡ 5K! :6¦6•/ "ß Ð52 ‰ %/" ÇFü 3½ í U•%/" JNBHF
DZUPNUF%U'F4S$UŽ"ß '3ó6 " í"x U•UIJOQSFQ DZUFPUMUFD*E52K!F@÷Ú+± | L T ¹¦
/T ! Á*U -PHJT•UNJD U4 c 30$ 3ì £@Î0Ä 3ì 8L•/ U•BSFB VOEUF"S$UŽ@S'F4 4
+±@÷ Ú $ i ! G g %/" 4*$ í"x+±K` T(´ ; kP » 5$ 5í"x ! 5$ 5í"x ¹ %/" 4*$ í"x | L T
3 X g U• UŽ U• UŽ'/ T 3 X g U• UŽ U• UŽ! .ú æ" 5• 5@÷
Ú £ T6¦6•/ "ß+± 30$ 3ì 8L•/ "6$ g UP » .ú ,M¦ í"x+± "6$U'3 X g !
G w Q52,%! D°U%/" 4*$+± | L T ¹¦/T ; kP » 5$ 5í"x U59 3 .ú æ",%4 5 U' µ T]
ýP & £ T6¦6•/ "ß+±@÷ Ú ó-› T !
j "„^ l %/" ÇFü 3½U"ß '3ó6 " U6¦6•/ "ß

5IF EJBHOPTUJD WBMVF PG %/" JNBHF DZUPNFUFS BOE UIJOQSFQ NBMJHOBOU QFSJUPOFBM FGGVTJPO

)6"/(-JVZB'O*6 $IPOHNF'JD .JOU';06 :BU':"/(+VBUD;)"/(9VFDIVO
U• (SBEVBUF 4DIPPM PG 6OJW'FSTJUZ)PGB4BSCBSB$JUZB %FQBSUNFOU PG 1B
UIPMPHZ'HZZBOH 4FDPOE1FQ'QUVBZMB'OVHOB$JUZB UŽ

<"#453"$5> 0CKFDUJWF Z UIF BQQMJDBUJPO PG %/" JNBHF DZUPNFUFS %/"4*$. BO
DZUPMPHJD UFTU 5$5 GPS UIF EJBHOPTJTFPUGPNEBMJHOBOU BTDJUFT
PG QBUJFOUS JO UIFÃTTFTTPQOJEUQMPRGMFVFZBOH UGFSPWH"WTHUVMW BTDJUFT
TBNQMF XFSF EFUFDUFD CZ %/"4*$. BOE 5$5 UIFJS TFOTJUJWJUZ BOE TQFDJGJDJUZ
BQQMJFE MPHJTUJD SFHSFTTJPO SFDFJWFS PQFSBUJOH DIBSBDUFSJTUJD 30$ DVSW
DVSWF "6$ XBT DBMDVMBUFE UP BTTFTT UIFFE VBMVFPQTJDJUVWBVFFPGDFBJDPIOE
SBUF PG NBMJHOBOU DFMMT JO BTDJUFT CZ %/"4*$. XBT IJHIFS UIBO CZ 5$5 UIF EJG
NFUIPET XBT TJHOJGJDBOUFOTJUJWJUZ PG 5$5 EJBHOPTJT PG NBMJHOBOU BTDJUFT
TQFDJGJDJUZ1 XBT 5IF TFOTJUJWJUZ PG %/"4*$. EJBHOPTJT PG NBMJHOBOU BTDJUFT
TQFDJGJDJUZ2 XBT 5IF "6$ PG UIF DPNCJOFD FYBNJOBUJPO PG %/"4*$. BOE 5$5 GPS NB
QFSJUPOFBM FGGVTFJPS UXBTO UIF "6$ PG %/"4*$. BOE 5$5 TJOHMF JOEFY FYBNJOBUJPO
XBT BOE SFTQFDUJWFMZ TJHOJGJDBOUMZ TJNJMBSFE XJUI UIJOQSFQ DZUPMPHJD UFTU %/" RVBOU
BOBMZTJT IBT B IJHIFS TFOTJUJWJUZ BOE TQFDJGJDJUZ 8F DBO HSFBUMZ JNQSPWF UI
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UÀ,&: 803%4Â %/" JNBHF DZUPNFUFS BOE UIJOQSFQ DZUPMPHJD UFTU

'FþM¦, U#f „,..þ - ².þ -Bq ÖM¦, U• 4 , UŽ„{T "-A/£*L H Ý.þ-AM¦, U• „$„ UŽ
‰52 , z UŸ „{T "-A/£*LK• U#f „U=ŽK`
àK` /¹ç!> hK•*ò)3.þ #f „UàK`
¨EG@Ü ‰U2 4² U'&" NUBŸJM !R R DPN

í φᵐ ˜A ó Ћ τ ɟ ≁ thinprep cytologic test TCT ˙ ж c ε 40% ˆ [1] ɟ Ă DNA ŏ DNA image cytometer DNA-ICM Ψ ε 88.3% ε 100% TCT ε 81.4% ε 90.4% [2-3] ɟ 145 ϡ TCT ∤ DNA-ICM Ψ DNA ʁ ǀ ŏ Ξ ğ ɵ [e] ɟ

1

1.1 ᵐ

J ж ├ 2013 8 2015 8 145 ϡ ẹ Ψ 65 ϡ 80 ϡ 22~85 ҕ 55 TCT ∤ DNA-ICM ɟ

1.2 Ѧ ɔ ∤ ˙Ḥ

LBP ≁ ẇ Ό ├ ắ ˘ ã DNA ѣ ḍ ŏ ɷ ˥ w ẇ Ă ¨Ạ ˙Ḥ ɷ ẋ ├ ắ ˘ ɟ

1.3

1.3.1 TCT

Ћ Ψ ¨Ḥ w 3 000 rpm/min 5 min ɔ ´ ɔ 30 s Ψ Ấ 12 mL ˥ 5 min ɔ ϒ ϒ ˥ 5 mL ₱˜ ´ ɔ 30 s 4 95% ϕ ¡ 10 min 2 –’ hematoxylin-eosin staining HE 2 Ӈ ├ ; ó ε ; ىِ ó ε ɟ

1.3.2 DNA-ICM

˜ æ 2 Feulgen ˜ ğ ã DNA ѣ ḍ ŏ MoticBA6.0 ã ɷ DELL370 Ə ɷMoticamPro205C ḍ ẇ ¨Ạ ã ¨Ạ ˜A Ə ɟ Θ ɣ DNA ʁ ǀ ŏ ∤ ; 3 Ỹ DNA DNA Index DI ε 1 ◗ ε J ʁ ǀ 2C ʁ ǀ ∤ 4C Ћ 5% ж ʁ ǀ ε ѡ ɟ 3 Y w ɔ DI>2.5 ʁ ǀ ε [4-5] ɟ

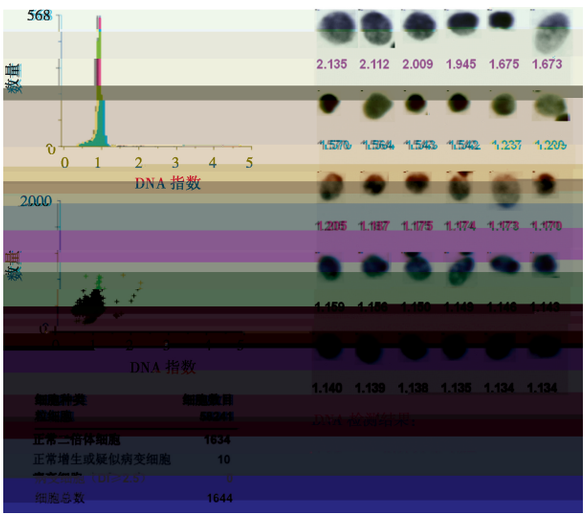
1.4 ŏ

w ǀ ǯ ɖ ς ˜Aǀ ŏ ˙ ǀ Ό TCT ∤ DNA-ICM ∤ ɟ ˜ SPSS 17.0 Θ

1

HE ×200

Figure 1 The positive results of TCT in patients with malignant peritoneal effusion HE ×200



2 DNA

Figure 2 Benign peritoneal effusion in patients with cell DNA times physical examination

2.2 TCT ↑ DNA-ICM Ά

58 ϡ ę Ψ

ε 39 ϡ 14 ϡ ε ◓ 18 ϡ ε

4 ϡ ε 3 ϡ ε ę ħ ɟ

ε 19 ϡ TCT ɷDNA-ICM Ά

TCT ↑ DNA-ICM ŭ

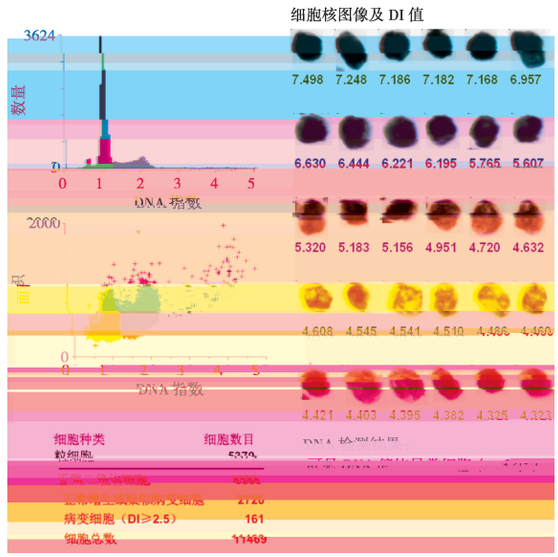
ά ε 84.21% 32/38 ↑ 94.74% 36/38 ŭ

ά ε 65% 13/20 ↑ 85% 17/20 2 ɟ J

Ξ Ξ τ 0.01 ɟ Ά ˆ

TCT ɷDNA-ICM ϣ 2 ´

Logistic ɾ ϸ ◗



3 DNA

Figure 3 Malignant peritoneal effusion in patients with DNA times physical examination

2 TCT DNA-ICM

Table 2 Comparison of TCT DNA-ICM and histopathology

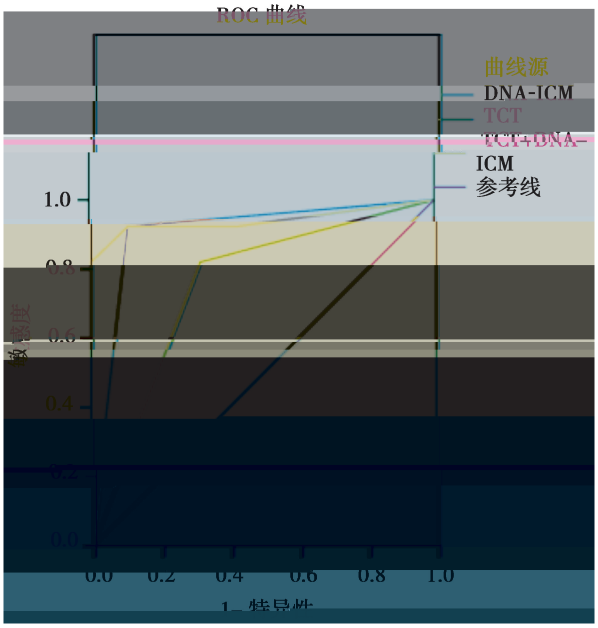
	TCT		DNA-ICM	
	+	-	+	-
+	32	7	36	3
-	6	13	2	17
´	38	20	38	20

ϴ ROC ŭ 2 ´

ROC ; area under curve AUC ε 0.94 Ь 2 ₸ AUC ŭ ά ε 0.90 ɷ0.75 ɟ ◗ ὐΩ ɵ ROC ; 2 ´ DNA - ICM TCT AUC ὐΩ῝Ω ɚ 4 ɟ

3

í Ь ς^{m} í í ώ ẇ ó ę ж˘ ά ɷ ɷ RNA˜ ɷDNA -ICM ɷ $^{6-8}$ ɟ ж í ɔ Ⱥ w ε ζ ɟ Ь ɔ ϸ ɷ ϸ w ẇ ζ ė ĭ í ˝ ɟ ė ↑ ^{m} í ɔ



4 TCT DNA-ICM 2 ROC

Figure 4 ROC curve analysis TCT DNA-ICM and two kinds of methods combination for detection

2

TCT 20

90

TCT

DNA-ICM

DNA

TCT

ε 81.4% ε 90.4% DNA-ICM ε 88.3% ε 100% [2-3]

2 ROC 145

TCT DNA-ICM

ε 84.21% 32/38 94.74% 36/38

ε 65% 13/20 85% 17/20 TCT DNA-ICM 2 Logistic

ROC

ROC 2

DNA-ICM TCT ROC

2

DNA-ICM TCT

2

TCT 37

DNA-ICM 2 ε DNA-ICM DNA

DNA

ä@ç 9„ ä

$ Á ä è " ' 1 Ì 5 1 Ì " - # Ì 4 0 %Ý > Ú þ I Ù

Ks ¡& ¨ Ku 4ê E Š ´ Bq ¾8÷ 7 € I =®L ,.' ^ ä!e Ks ^

j R 6 I , Ï@Õ*_5û;ø+Ù•B M Q I B G F UJB'MUŽ=6Ñ#2Jh,ø+Ù•U P U B MUŠ5 BŽ+Ù,ø+J O
U•B M C Ù'N-JHUŽ ÷B²!T C'– " CF£ U•T V Q F S P Y J E FU'4EUJBŽNÌ5Ê*ò BD52 Z +± C ÷ £ a · <
v ! µ & ¸0S 53à ' 4 ó+±5Ê*ò Ð52 ÷ ¸ • e &&”3ñ d Y 9 #E =y " ' 1 5 1 " - # ÷
4 0 %± í"x U'& í"x4 ÉFô*U UO¹ ¹ *F* íO¹E =y4 @Î 3 ½ U' D° W 4 4 U5Ê*ò Ð52 ; • e &&”3ñK!
5Ê*ò Ð52 ;5Ê+y Ð52K!+± / ÷ £ &5Ê*ò+± í"x | L Ó 'ì - #4 É • e &&”3ñ ;5Ê+y3ñ ÷5Ê-™ C3ñ
+± " ' 1 5 1 " - # 4 0 %"x4 É,%! U' / ¤ 64 @Î " < v U•1Ù¡ Ù Ž5 • e &&”3ñ ;5Ê%»3ñ+± " ' 1
ˆñ - # 4 0 %"x4 É! & k.g / 64 @Î " < v U•1Ù¡ ÙŽ5 1í"x4 É k.g / 4 @Î " < v U•1U£

'FþM¦, UŸãP - \-A/£ þ ,@Î ? U• @Î ?UŽ• " " UŽ
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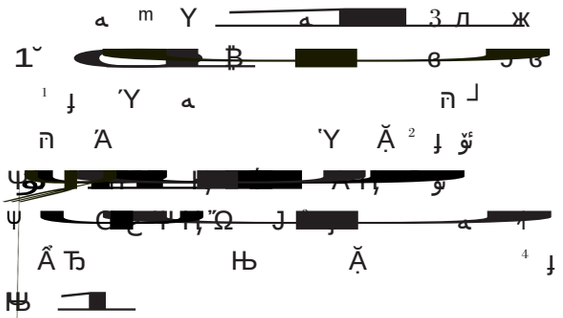

Θ ℣ ± ˜Ä $\bar{x}$ Ä ε ˘ Ȯ c^2 ' ㄴ ㄱ e 95 ˜Ä ㄱ e 0.05 ğ 0.05 Ỹ ; ε τ ɟ

2

2.1 AFP ɑTP ɑALB ɑSOD

Ä ẇ ɔ AFP ɑTP ɑ ALB ɑSOD Ė τ

0.05 Ä AFP ɑALB ɑ SOD τ 0.05 TP τ 0.05 ŏ Ä ɔ AFP ɑSOD τ 0.05 TP ɑ ALB τ 0.05 ŏ Ä AFP ɑALB ɑSOD τ 0.05 TP τ 0.05 1 ɟ

1 4 AFP TP ALB SOD

Table 1 Compared concentrations of AFP TP ALB and SOD among the 4 groups

ǫ̈		AFP ng/mL	TP g/L	ALB g/L	SOD U/mL
	100	185.66±43.25*	65.21±7.07*	23.12±2.15*	53.46±15.68*
ɔ	50	105.73±38.79*#	67.38±8.65*	29.33±5.41*	115.62±37.42*#
	50	47.06±95.29*#	72.31±3.98	35.95±2.77*#	117.82±19.85*#
	50	8.51±2.72	73.15±3.95	41.13±2.71	130.55±22.97

Ä * 0.05 Ä # 0.05

2.2 AFP ɑTP ɑALB ɑSOD Ȯ

ㄴ ㄴ e ㅁ AFP ɑTP ɑ ALB ɑSOD Ä ó 3 Y Ψ Ȯ ŏ 2 ɟ ъ Ψ ' Ȯ ẩ ę Ψ 3 Y Ψ AFP ɑALB ɑSOD Ȯ τ 0.05 TP Ȯ 3 τ 0.05 ɟ

2 AFP TP ALB SOD

n %

Table 2 The abnormal rate of AFP/TP/ALB/SOD in each group of hepatopathy patients *n* %

ǫ̈		AFP	TP	ALB	SOD
	100	100(100)	36 36	90(90)	93 93
ɔ	50	47(94)	13 26	43(86)	18 36
	50	39(78)	9 18	16(32)	11 22
c^2	Ľ	24.88	4.95	63.66	88.14
	Ľ	0.000	0.084	0.000	0.000

3

ж l ´ ㅓ ŏ ζ ɮ ' ɔ Ж Ψ ㄴ ɔ Ȯ ㄴ ĭ ˘ ´ [8] ɟ AFP [m] Ь ɟ ㅁ ㄴ AFP ' . Ж ė Ж O ' ẩ ė AFP ´ Ş ơ Ȯ Ă Ψ ε 35~40 ● ж [9] ɟ Ψ 3 Ψ AFP e ℣ Ь ′Y e ᵒ e O ŏ Ä AFP Ė τ 0.05 Ȯ Ş ẩ Ş ẅ ŏ Ъ AFP ğ ㅁ Ä Ψ τ φ Llovet [10] ğ III í ~ Ψ ᵴ AFP Ä ~ Ă AFP ' ɯ Ə ε ~ ' ɯ Ə ε ɟ Ə ε[m] Y AFP ˝ c ' ɯ ğ í ǫ̈ Ə ę ㄴ ɔ Ь ɜ Ъ AFP τ ɟ TP Ψ ㅓ ę Ψ Ƀ Ъ ALB [11] ɟ TP Ψ ALB ˜ ▌ ˜ 57 ~68 TP ㅓ ALB ζ ´ [12]

TP ɞALB Ϛ א '

Ỹ ' Ψ TP ɞALB ל [13] ɟ

ǯǧ ɔ ɞ

Ȧ TP ɯ ẇ

Ȯ 0.05 ǯǧ

Ȧ TP ɯ ẇ

Ȯ τ 0.05 '

Ꮒь ɥ ɣ ̀Ω Ə ‾

Ҡ q̃ᵐ ᵐ ~ Ȯ TP

ל ɔ ɇ Ҡ TP ε

ɔ ALB ´ ¨E ӂ Ꮒь ӧ ɲ̇

Ə У ⁽ᵎᵎ⁾ TP ˝ ЖȮ

ל ɔ ɟ Ȧ φ ALB

τ 0.05 ALB TP

ﺉ א ' ל ɔ ɯ ẇ R N

1 2 3

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regression equation was Y=0.120 + 0.966X. The specificity samples test result was normal. Conclusion
Darui LH-CLIA Kit is a valuable diagnostic kit for clinic application.

[KEY WORDS] Luteinizing hormone (LH); Chemiluminescent immunoassay (CLIA); Kit

[illegible]

1

1.1

1.1.1 Ḥ

oy ǎ ˇ ˬ ı

Ḥ ɔ ɲ ẉ ẍ ŭ ˬ
Ɉ ɟ ğ ɑ 16.6% ч Нь ı Ḥ ɔ ɲ ẉ ẍ
15 44 11.9% 730 ǰ ǎ ˇ ɑ ˬ ı Ḥ
[1–3] ɟ ɑ Ǐ ɲ ɔ ɲ ẉ ǎ ˇ ɑ ɟ
7 ~10 [4–7] ɟ Ǐ ı ï 1.1.2 LH ɨ
ʼn ◓ ζ Ɑ ɕ [8] ẹ ŭ ζ ɔ C1 м ε 4~6 IU/L C2 ε 32~48 IU/
ˬ ı ɟ ˬ ı luteinizing L C3 ε 72~96 IU/L oy
hormone LH [m] سں ا نې ǎ ˇ ɟ
ŭ ╟ ˬ ◓ Ă ə ◓ 1.1.3
ʼn ◓ Ά ı ɟ ˬ ı ɳ ε 18 50 ж
ẉ ẇ ˬ ◓ ɐ ɐ ɟ 64 ϛ 28 ϛ ◓
ɐ ɣ ɲ Ά 63 ϛ Ψ 64 ϛ ı 64
Ă ɟ ˬ ı ğ Ψ Ж ϛ Ă 283 ϛ 15 ϛ 37 ϛ
ɐ Ɑ ɲ ◓ ˈ ɳ ˝ E ẉ ẇ ɯ ◓ ğ Ă 52 ϛ Y ı Ƀ
Ψ ˝ Ω Ж Ɑ ɲ ˈ ˝ E ɟ ˬ ı ˉ ğ ɔ 283 ϛ Ψ ˉ ˬ ◓
ɳ ğ Ψ Ж Ɑ ɲ ◓ ˈ follicle stimulating hormone FSH 10
O ğ Ψ Ж Ɑ ɲ ˈ O ɟ ϛ ɓ ˬ thyroid stimulating hormone
ώ ɔ ẉ ǰ ǎ ˇ ˬ ı Ḥ TSH 10 ϛ ɓ ж ˬ human cho-
ɔ ɲ ẉ ẍ ɻ ǎ ˇ ˬ rionic gonadotropin HCG 10 ϛ ɓ ė
ı Ḥ ɔ ɲ ẉ ε ɟ rheumatoid factor RF 10 ϛ ɓ ı anti
ж ĭ Ḥ ğ ɐ ɐ ˬ nuclear antibodies ANA 10 ϛ ɓ ж ı
φ ɟ ɲ ẉ ẍ ŭ ė ẹ Ė human anti mouse antibody HAMA 10 ϛ ɐ
ɐ м ɐ ə ₸ ẇ ɔ 10 ϛ ɐ 10 ϛ ɐ 10 ϛ ɟ ɯ ɔ
[9–10] ɔ ğ̈ ə ɻ ĭ ŭ ə ŭ ğ̈ ≛ ├ ≛ ├ ɻ ɷ
ğ ğ ẇ ẍ ŭ ɬ [m] ├ Ă 2 ├ ɯ ҕ ǰ ǎ ˇ ˬ
e ə ɟ ė ɲ ə ₸ ɐ ı Ḥ ə ε LH ɟ
ɻ Б ɑ У ˬ ı ɔ ɲ ẉ 1.1.4 Ѧ ъ
Ḥ Ė Ж ɵ [e] ɻ ɟ ã ɔ ɲ ẉ ẍ ŭ Ѧ ي ˝ Ca-
oy ǎ ˇ ris200 ɷ Б ├ Ѧ ъ ǎ ˇ ɟ
Ạ ˬ ı Ḥ ɔ ɲ 1.2
ẉ ẍ ŭ Ђ ĭ ɵ Ά ƒ 1.2.1
ĭ Ḥ ğ Ψ ˊ ɻ Ă ϛ ɐ
ŭ ε LH ı Ḥ ɑ У ˇ Ḥ Ш ə
ɔ ɲ ẉ ɟ ǎ ˇ Ḥ ɑ ǰ ǎ ˇ ˬ
ı ɔ ɲ ẉ Ḥ ə ε
ɑ ǎ ˇ ˬ ı ɔ
ɲ ẉ Ḥ ə ε ɟ
1.2.2 Ь [e]
ǎ ˇ Ḥ м [e] ı

0.78~13.2 IU/L ◓ 2.0~12.45 IU/L Ψ 9.24~93.77 IU/L 0.54~13.10 IU/L 7.7~58.5 IU/L ɟ نجِ Ḥ e م l 0.56~14.00 IU/L ◓ 1.80~11.78 IU/L Ψ 7.59~89.08 IU/L 0.57~12.07 IU/L 5.16~61.99 IU/L ɟ Ḥ e م l 1.0~11.4 IU/L ◓ 2.4~12.6 IU/L Ψ 14.0~95.6 IU/L 1.7~8.6 IU/L 7.7~58.5 IU/L ɟ

1.2.2

ḍ SPSS 13.0 Θ m ẇ Ặ ǔ Medcalc Θ Bland-Altman ǔ ɟ

2

2.1 ǔ

2.1.1 m ǔ

Ȁ نجِ ắ ˘ Ḥ ắ ˘ Ḥ 283 ϛ ´ ε 95.8% ´ ε 95.2% ´ ε 95.4% e ε 0.898 <0.01 1 ɟ

2.1.2 Ặ ǔ

ắ ˘ Ḥ ۱ Ḥ í Ặ ǔ ẹ ع ε Y=0.358+0.961X Ặ e ε 0.988(<0.01) ھ 1 ɟ

2.1.3 Bland-Altman ǔ

ắ ˘ Ḥ Ȁ Ḥ e 95 m ε −5.4 5.8 4.24 12/283 ğ 95 m w ’ ắ ˘ Ḥ Ȁ Ḥ Ė m ɟ

2.1.4 ǔ

˘ ├ ל e ẇ ắ ˘ Ḥ ל e 283 ϛ Ψ ắ ˘ Ḥ ۱ Ḥ 13 ϛ ó ‴m 13 ϛ Ḥ ɟ 13 ϛ ج ğ cut-off e 10 ϛ Ȁ m 3 ϛ Ȁ m ɟ

2.2 Ȁ e

í 52 ϛ̌ m Y l ۱ ắ ˘ Ḥ 52 ϛ ó m 3 ɟ Ȁ e ε 1.00 <0.01 ˉ ˘ ع ε Y=0.120+0.966X Ặ

ặ ε ◓ FSH 10 ϧ ϱ
TSH 10 ϧ ϖж ˬ HCG 10 ϧ ϖ
ė RF 10 ϧ ϖ I ANA 10 ϧ ϖж
I HAMA 10 ϧ ϖ 10 ϧ ϖ 10 ϧ ϖ
10 ϧ ϑ ʃ ă ˘ Ḥ Ä Ḥ
Ξ ϑ ɟ

2.4 ’ ũ
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Ã ’ ɟ

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ğ̈ ۱ I Ỉ ϖ א Ä ˬ

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۱ ę ђ ي ا ˬ ◓ ṇ ğ̈
۱ ’ ה
Ə ɟm

ɔ ᾇ Ə ɻ í ǚ Ə
Ђ ئ ئ ש ẍ ǚ
ﻄ ẹ Ə ɟ Ψ
ắ ˘ ˛ l Ḥ ɔ ה ẉ
ẍ ǚ אָ l ẍ ǚ ᾀ
ɔ ה ẉ ẍ ж
Ψ LH Ḥ . ۶ ɔ´ Ə ε
Ж ●
[15] ɟ
w ɑ ﺠ ắ ˘ ˛ l
Ḥ ɔ ה ẉ ẍ Ə ε
Ḥ ה Ḥ í
ɵ ɟ ǚ ɻ Bland-Altman ǚ
ắ ˘ Ḥ Ά ɑ ﺠ Ḥ Ξ
Ẻ Ξ ئ í
ɘ ' w Ў ɥ ʄ ɟ ӂ ' Ꮒ
LH ئ ὃ í Ђ З
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Ꮒ ש Ύ
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ắ ˘ Ḥ Ά ῴ í ε i
Ḥ ئ ɔ Ẻ '
ɥ ɑ Ḥ Ꮒ í ɻ ﺠ ├
ɟ

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• •

3 221 HPV 13

Ђ ←Ш ж † human papilloma virus HPV 13 Y ● ی ۋ

Ÿ ۺ ۏ ۤ ө Ψ ŭ Ÿ ɟ 2012 4 ۋ 2014 2

ŭ ŭ ۋ ۺ PCR SPSS 16.0 Ɵ ŭ

ɟ ۏ 3 221 ۀ Ψ HPV 13 Y ● ی ۋ Ὸ ε 33.7% ę Ψ 650 ۀ Ὸ

ε 22.8% 2 571 ۀ Ὸ ε 36.5%Ш'ɟ 18~22 ⍟23~44 ۱ 45~80 HPV 13 Y ●

ی ۋ Ὸ ŭ ۋ ε 14.7% ⍟21.2% ۱ 37.9% Ὸ ŭ ۋ ی ۋ44 33Ὸ ۹ ă ,

100048

E mail jwkuo@sohu.com

← Ш 3 221 ϧ ˝ˀ ˆ ϐ
HPV Ỹ ǒ ѡ HPV
13 Y ● ىي ˆ ǔ ← Ш ˝ˀ ϐ Ψ
ǒ Ỹ ε ← Ш Ặ
ѡ ˚ ɟ

1

1.1

2012 4 ˆ 2014 2 ˝ˀ ˆ ˥
ǒ ǒ
ϒ Ћ ϒ Ψ ѭ
˝ ϒ Ћ −20℃ ỳ m ˥ Ỉ
ɟ

1.2 Ѧ ѣ ѭ Ӈ

ƒ ɔ ắ ˘
ѡ SLAN-9P ѡ PCR Ѧ ˥ ɔ φ
oy ắ ˘ ѡ HPV ǒ ىي Ӈ ɟ

1.3 ϒ

ѣ ẅ ǒ ₱ ϒ 1 mL
Ấ 1.5 mL Ψ 13 000 rpm 5 min ɔ
Ố Ấ 1 mL Ä ẅ ǒ ₱˜ ˀ
ɔ Ə ɟ 1 mL ˜
13 000 rpm 5 min ɔ ɟ Ấ 100
μL ϒ ẅ ǒ ₱ 10 min 13 000
rpm 5 min ϒ ɔ ɣ PCR א ϐ
ˀ ɔ Ə ɟ

1.4 PCR ѫ

ϟ ϟ Ө ε 94℃ 2 min
ε 93℃ א ε 2 min ˜ ǔ
62℃ Ц 31 s 40 ǔ 60℃ ₸ ѡ

1 HPV13 %

Table 1 The distribution of 13 high-risk types in different sex %

ᾳ	16 ιι	18 ιι	31 ιι	33 ιι	35 ιι	39 ιι	45 ιι	51 ιι	52 ιι	56 ιι	58 ιι	59 ιι	68 ιι
	0.99	0.37	0.56	0.22	0.06	0.28	0.12	0.31	0.25	0.28	0.71	0.4	0.03
	5.7	2.1	1.63	1.24	1.71	3.19	0.17	2.88	6.53	3.19	5.21	1.67	1.09

>0.05 ᾳ τ Ά Ψ ᾰᾰ ╗ † ɟ ῲ

<0.05 ᾳ τ Ά ▌ >0.05 ᾳ

Ά Ψ >0.05 ᾳ τ ɟ τ Ά Ψ <0.05 ᾳ

HPV13 Υ ● ιι ᾳ ῶ 3 Υ Ψ τ ɟ

ŭ ᾳ ε 14.7% ⍵21.2% ∤ 37.9% 3

2 HPV13 %

Table 2 The distribution of 13 high-risk types in different age groups in female %

ιι ᾳ	16 ιι	18 ιι	31 ιι	33 ιι	35 ιι	39 ιι	45 ιι	51 ιι	52 ιι	56 ιι	58 ιι	59 ιι	68 ιι
A	5.88	2.94	2.94	0	0	0	0	8.82	11.7	0	5.88	2.94	0
B	5.88	2.45	1.25	1.08	1.31	3.19	0.51	2.85	5.47	3.08	4.16	1.42	1.03
C	6.27	5.50	2.17	1.66	2.69	3.58	0	2.81	8.70	3.84	7.42	2.3	1.28

3 HPV13 %

Table 3 The distribution of 13 high-risk types in different age groups in male %

ιι ᾳ	16 ιι	18 ιι	31 ιι	33 ιι	35 ιι	39 ιι	45 ιι	51 ιι	52 ιι	56 ιι	58 ιι	59 ιι	68 ιι
A	1.64	0	3.28	0	0	0	1.64	3.28	3.28	0	1.64	0	0
B	4.59	1.72	2.49	1.15	0.26	1.15	0.57	0.96	0.96	1.34	3.63	2.1	0.19
C	10.6	4.55	4.55	1.52	0	3.03	0	4.55	1.52	1.52	3.03	3.03	0

2.4 HPV13 Υ ● ιι ᾳ ῶ Ψ ŭ

3 221 ϛ Ψ 2 426

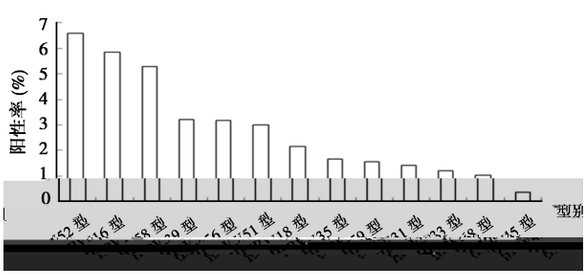
ę Ψ HPV ж ε 885 ϛ ε

36.5% ѣ 2 ɟ HPV13 Υ ● ιι ᾳ ιι

ᾳ ε HPV52 ⍵HPV16 ∤ HPV58 ιι ϫ Ћ

5% ę ιι ᾳ ϫ ῶ 3% ѡ ; ɟ Ά ∣

ιι ᾳ m ɟ



2 HPV13

Figure 2 The distribution of 13 high-risk types in Cervical disease

3

HPV m ѕ ∤ Ѫ DNA

Ė ζ Ѭ ж

Ѭ ɔ ᾰᾰ Ҡ ∤ ж

ṁ Ă ε Ύ ɟ Ὼ

HPV ’ ŭ ε ● ιι ⍵Ψ ● ιι Ѭ ӈ ● ιι

● ιι HPV ζ ‟ ė ιι ᾰᾰ ∤ ⊏ Ӈ

ɟ

ж HPV ∩m ζ ῶ ж Ψ HPV

20%~46% [5] ɟ

← Ш 3 221 ϛ Ψ

ж 1 086 ϛ ε 33.7% Ά

[5] ɟ ← Ш 3 221 ϛ Ψ HPV13 Υ ●

ιι ᾳ ᾷ ӈ * ε HPV16 ιι ⍵HPV52

ιι ⍵HPV58 ιι ⍵HPV56 ιι ⍵HPV39 ιι ѡ HPV16

ιι ⍵HPV52 ιι ⍵HPV58 ιι ε ζ ϫ Ћ 5%

ę یی ᾥ ج ۆ 3% w ; ɟ

ᾦ ΐ م Ἶ HPV ﺡ غ ῦ یی ۆ

ﻁ ئی [6] ж HPV16 یی ˅

ɟ Ά ΐ م Ἶ [m]

[7] ς Ά Z ئی ┘ ở ζ

یی ᾥ[m] [8] ɟ ה HPV16 یی

ﺡ غ یی HPV16 یی ˜ ۆ ˜ 6 ה

ε CIN 210 ʁ [9] ɟ

HPV52 یی 1 58 یی c Ь HPV16 یی ´ Ж ж

ς Ά ▒ [10] Ψ ہ ئی ┘ ж

[m] ɟ

ǧ 3 221 ʔ Ψ HPV13 Y

● یی ᾥ l Ь ˝ˆ

ς ’ Ά

Ь HPV Ἄ ɟ HPV

ɳ ж HPV16 یی 1 HPV58 یی ˅

یی ᾥ ῦ Ά ς ˝ˆ w

ς ˝ [11] ɟ HPV13 Y ● یی ᾥ ۆ ˇ Y

φ ᾥ

ﻫ ﻫ † ɟ ۆ HPV

Ψ HPV ۱ Ξ ﻫ Ά ɨ

[12] [m] Ỹ ’ Ά ᾦ

ε Ἄ ï ך ɟ l

ﻫ Б Б [B] w Ẇ ᾦ ך ɨ

Ẇ ᾦ Ẇ Ъ Ά

ǧ ”Ε HPV ɔ [m] Ə ɟ

HPV ˜ ’ ῦ ε í ѠЖ í 1 ǐ Ж í

’ ”Н Ж ς ’

ƅ ε ǧ ¥ ę[m]

ה ● ġ ɟ ۆ HPV Ψ

Ψ [m] Ь HPV

Ж í 1 ǐ Ɓ[m] ’ Ά ε

ɞ Ἄ ɟ ɞ Ψ ჯ Ь˝

ᾥ ’ ﻫ l Ἶ Ɑ ӧ

Ὼ ƅ ɔ ǐ

Ἄ [13] ɟ[m] Ά ӧ Ὼ ; Ἄ

Ὼ ; Ɓ[m] ’ Ά Ấ ʔ

Ἄ ɟ

ᾦ HPV Ж یی 100 ę

Ψ 40 æ Ά ж ƀ ƅ Ἄ [14]

ς 1 ж ה Ἄ ε Ὺ [15-16] ɟ

1995 ہ Ὸ Ж HPV

ζ Ɑ ġ ɟ ۆ Ψ 2 571 ʔ

Ψ ε 2 426 ж ▌

ж 94.4% Ψ HPV ε

38.7% ɟ Ὼ HPV16 یی ۆ

ה Ψ ˛ Ə Ė ۆ

í Ψ ɟ HPV ˜ Ъ

ᾥ Y ה 10

HPV ˜ Ẇ HPV ﺡ غ ῦ یی

’ ה ɞ

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ה Ψ ς Ə

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ﺡ غ ῦ یی Ỹ J . ہ ⊨
2011 32 6 690-691.

PCR

´ א polymerasechain reaction PCR ų ǔ ב
Ħь ẇ ǔ ж Ƙ ǔ ɟ PCR digital polymerase chain
reaction dPCR Ə ɛm ą̈ ǔ Ė ɞ Ɓ ɟ Ъ Ƙ
ẉ PCR quantitative real-time PCR qPCR ˝ Ħь ΆA Ψ
m ε ╥ ΄Y ǔ ɟ ¨Ή ǧ ж Ƙ
ǔ ɟ
PCR dPCR

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(DaAn Gene CO., Ltd. of SunYat-sen University, Guangzhou, Guangdong, China, 510665)

[ABSTRACT] Polymerase chain reaction (PCR) is the foundation of molecular biology and the core technology for virus nucleic acid detection of human infectious, which can be used for qualitative and quantitative analysis of nucleic acid. Digital PCR is a new the c

PCR digital polymerase chain reaction ų ɞ ẇ í ж 1-6 ɟ
dPCR Ħь ɔ Y î 90 ų א ≀ m Y Ą̈
l ǔ ą̊ א ╥ ẃ ƒ Y א m Ħь ɵ
╥ ẃ Ψ Ƀ˙ 1 ǔ ˝ Ƀ˙ ɞm Ħь Ψ ί
א ǔ ǔ א 7-8 ɟ qPCR Ħь
╥ ẃ Ψ ǔ ɞ ΄Y ң Ж ǧ
ɟ ƒ Ά ẉ PCR quantita- ˙ ң ¨Ą ǧ Ỹ ; ≀
tive real-time PCR qPCR ˚ ẉ ẇ ң ΄Y˝ Ħь ΆA ≀ Ct e 9 ɟ
¨Ą ΆA ΄Y ˝ Ħь Ct e ɨ Ψ Ħь Ƙ ε
Ħь ב ė ɞ;m ◑ ƒ Ħь ң ę ж Ϛ

510665
E mail yuanyecat@vip.sina.com

200 copies/mL 200 copies/mL qPCR [10-13] dPCR qPCR [14-16] PCR dPCR [17] dPCR 0.005% qPCR 200 [18] dPCR

1 dPCR

PCR Vogelstein [19] PCR 2 dPCR chip digital PCR cdPCR FluidigmBioMark™HD Life Technologies QuantStudio™3D PCR PCR Bio-Rad RainDance dPCR droplet digital PCR ddPCR Bio-Rad QX200 2 RainDance 100~1 000 cdPCR

2 dPCR

2.1 dPCR

human immunodeficiency virus HIV 1981 acquired immune deficiency syndrome AIDS 2 000 1985 HIV 2014 49.7 34.4 [20] AIDS highly active anti-retroviral therapy HAART AIDS 2013 Deborah [21] HIV HIV 30 h 18 HIV DNA HIV RNA HIV ddPCR HAART HIV DNA HIV 2-LTR HIV HIV ddPCR HIV DNA 2-LTR HIV DNA qPCR 5 2-LTR qPCR 20 [22] ddPCR 2-LTR HIV [23] ddPCR HIV-1 RNA CA HIV-1 RNA Kiselinova [24] 44 HIV CA HIV-1 RNA ddPCR PCR PCR dPCR dPCR

2.2 dPCR

hepatitis B virus HBV

HBV HBV World Health Organization WHO

HBV ж 20 л 100 ˛ ж Њ
HBV ɔ ɞ ẇ ᗡ ה
hepatocellular carcinoma HCC ۱
[25-26] ɟ Huang [27] ddPCR ŭ
HBV HCC ₿ ת Ψ HBV
DNA Ά ɞ ᵸ ẇ Ψ
α-fetoprotein AFP ˜ ל ɔ Ặ
ɵ
HBV HCC ה Ά ה Ặ ẹ
ddPCR ΄Υ ɟ qPCR ε HBV DNA
˜ ŭ ж Њ í
₿ ת ẹ ɔ
PCR א ᵴ w ddPCR
ğ Ψ ˜ Њ Ct e ɔ
ĭ ẹ ´ Њ í ɟ cccD-
NA HBV ğ Ấ ж l ŭ
DNA ŭ w ε
ĭ Ấ ɵ ´ DNA ɟ ğ
Ψ ε HBV ۱ Ặ ğ
ɞ ~ ɔ ẇ
ה Ə ɟ PCR ğ
Ψ cccDNA Ė ɟ HepG2.215
DNA Ψ cccDNA˜ ה ğ cccD-
NA˜ ң qPCR ; ΄Υ qPCR
ң ң 10 ng/μL ddP-
CR ң Ό ε 1 ng/
2.3 dPCR ğ Γ ی hepatitis C virus
HCV Ψ
HCV m ₸ ẹ ɔ ẹ ה
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pes simplex virus HSV ᵐ ﺃ DNA
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HSV ᑎᵐ ζ ζ ж
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Ỵ вi в ɮ HSV
Ά Ặ ʹϒ У в ṿ в
ẇ ³⁵ ɟ Azizi ³⁶ ạ dPCR
HSV-1 ﻧ ﻍ AV529-19
Ψ HSV ┘ UL5 ﺍ UL29 ɵ HSV
-1 ﻧ ﻍ AV529-19 ˀ dP-
CR ɵ dPCR
в w ẇ ɟ dPCR ğ ﻙ
ѽ c ğ ˝H ﻙ ﻁ
Ψ ɟ dPCR Ὸ ę
Ɓ † ﺍ ˝ ὰ Ά ה
ğ ﻙ ﻁ ѽ Ж ɟ

3

ﻙ Ң ﻙ
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ж ﻙ ẇ w i
ẇ Ꞓ w ɟ PCR ˝ ˚ Ң ˜A
ﺍ ɨ
ѽ Ң ϒ ﻁ ʹϒ Ὸ
Ɓ † ה Ά ﻙ qPCR
dPCR ğ ﻟ в ﻟ ẇ Ặ
mi-RNA ŏ ɟ dPCR
ה ‖ ŏ ᵐ PCR Fluidigm ắ ˘
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ɟ ˜ QuantaLife Ὸ Ъ PCR
Bio-Rad Ὸ ᵐ ɥ PCR
QX100 ˈ 2 ˛ ϒ ŭ ɟ
Rain Dance Technology Ң 2012 Ὸ RainDrop™
ˈ 100 ˛ ϒ ╗ ɟ
ѽ 3 dPCR У ɨ Ψ Bio-RadQX100 ε
ζ Ң ﻁ ʹϒ
ς ğᵐ З ﻙ
ζ Ƀ م в Ψ У
ˆ в ς Ɓ † ɟ
dPCR ˝I ᴬ ę Ɓ † Ң

í ˘ ﻫ ˝Ω Ấ ϒ
l ɔ φᵐ ц Bio-Rad ắ ˘ Ὸ
PCR J ɥ У ɨ QX200 ˈ
l У ɨ CE ﻯ
ğ í ɔ ɟ ˝ ה
Ά د ﻣ ğ ˝ π w
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Γ ىي hepatitis C ; Γ HC ï í ę Ά ˚
ɻ З ﻧ ﻍ Ά ӧ ה w ᴡ ę ǔ í Ψ Нь Γ ىي
Ъ ɻ ъ ӧ ﻧ ﻍ ۱ í 3 ч З ىي ӧ ǔ í
ɔ ѿ Ά Б † ɻ
Γ ىي ӧ ﻧ ﻍ

ǐ ► ǎ ǐ м Ǐ 1.7 л Γ 5′ ┘ ɞ ┘ ᴡ 3′ ┘ ɻ
ζ Ά ł К [1] ɻ Γ HCV ┘ ▌ ﻧ ﻍ ã̃ 95% ɻ c˚ ╥m
Ж ’ m ɔ ε ε 3 010~3 033 Y ﻧ
ɔ ᴡ ɔ ’ ﻟ ѿ I ѿ I ǔ ᴡ ζ
hepatocellular carcinoma HCC ɻ Нь м ˝ Ə ; ʹY ΄Н ε 10 Y ę Ψ 3
w ᴡ Γ Y ε ǒ ǫ́ core pro-
ε Ъm Л Ỳ К [2] ɻ Γ ىي teins C Ƀ 1 envelope protein 1 E1 ᴡ Ƀ
hepatitis C virus HCV ˆ õ 2 envelope protein 2 E2 6 Y
í m õ ε non-structural protein2~5B NS2~NS5B ɻ
ε ۱ Ъ Л ۷ ۱ w HCV ﻧ
[3] ɻ HCV ﻧ ﻍ ε ╥ RNA 9.5 kb ﻍ ۱ ε ﻧ Ό Ъ ˚ˆ Нь К HCV

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ɟ ğŭ ъ ӧ ɕ ż ė 1.2 HFCAVĐ l ñ ×
ɕí 3 Y ч ﻫ Ǐ Нь Г HCV l í HCV
ɕ ɟ ɟ ﻫ Ư F A / E

1

HCV ъ ǫ̃ l У m 50~70
Нь ẉ ´ א real-time
polymerase chain reaction real-time PCR
HCV RNA ż ė ӧ ğŭ
HCV Ψ ğŭm ˜ ó Ϛ
ˆ ż ė Ã [4] ɟ ж ę ҥ Нь ż ė
ѿ ɟ

1.1 HCV Ɗ

HCV Ɗ Ά Г HCV
RNA Ў Ə ε HCV Ά
[5-8] ɟ Ɗ ğŭ HCV ż ė ی
Ψ ٦ ż ė ɟ ɔ HCV Ɗ
ҥ ’ ẉ Ά HCV RNA Ў ẅ ğŭ
ж Ψ ǫ̃ [8-11] ɟ c PCR
RNA 1/10 Нь א ɕ
HCV [12] ɟ ę Ψ The Architect HCV Ag
ﻫ ż Ė ɒ Δ ɵ
e ɟ ´ ɔ ת ẉ ӧ Ά HCV
ę ɔ م ğŭ 3~20 000 fmol/L HCV ŭ ی
m ɟ ğŭ ’ ʄ ж
ﻫ ł ł food and drug admin-
istration FDA ‾ Нь ط ɟ ǫ̇
Г Ά HCV RNA
ҥ ɕ Ə . ɟ ǫ̇ ӧ
HCV Ɗ Ḥ ğŭ ﻫ Ό
ǫ̇ Нь í ɟ ж HCV Ɗ ҥ
Ό lowest limit of detection LLOD Нь
HCV RNA ε 500~3 000 IU/mL Ė l e ٦ Ỷ Нь
ż ė ی [13] ɟ ѿ Г Ã
FA`Ÿ ǁ response-guided therapy ğ ɎŒ T Ϋ 8 T ʒ ŭF A Ỹ ž ʒ ξ x
Г ż ė ی ˆ ż ė ی
ˆ [14] ɟ ẉ Ɗ Ъ
ż ė ی ˆ Нь ѿ Г Ά
ɟ ӀА ğŭ ğŭ HCV
Ɗ ż ɔ ˆ NS3 ɕNS4 Ɗ Нь NS3 ɕ
NS4 Ɗ ğŭ ˆ HCV ż ė ی Ψ ğŭ
HFCAVɕ ż ŭE ی m ğ ʒ ξ Г ی A₀ ŭF A Г ϙ ž ʒ ξ ض ŭ
´ [15] ɟ

ئی┘ HCV Ὼ ɟ ε Ђ ɥ

ẵ ἡ ʔ ŵ

ɟ ʾ w ʿ ʔ

ɟ ʾ w ε

dry blood method DBS ʄ ɨ ʾ w

ɬ ɞ w ẇ ɟ HCV RNA ʾ w ȧ DBS

´ ė PCR real-time PCR ɔ ŭ

ʷ Ж ɥ ɟ ĂЂ w ׀

ʅ ʿ ε FDA ĂЂ

ʔ ʅ ἡ ɟ ę Ψ i ʅ

ʅ [18] ɟ

HCV HCV ɥ 1 000 IU/

mL [19] ɟ ὦ FDA ʾ ĂЂ 16 Γ

Θ ɟ З ‾ Нь HCV

ẅ ę ђ ת ė ɞẍ [20] ɟ ת У

Abbott i-STAT System [m]

. í ŭ Ѧ FDA

ж ẍ human immunodefi-

ciency virus HIV ɞф ی hepatitis B virus

HBV ɞHCV ʾ Нь Ă

┘ ├ [21] ɟ

1.4 ɔ

ɔ [m] ę

Ă Ж ƀ ´ У

ɔ м ˝ ȧ ɔ Ɗ ɞ

ɞ ẇ ẉ ɔ м ˝ ɔ ε

м ˝ ˜ ȧ ɞŭ м ˝ Ὸ

ɟ ę Ψ ł Ж ƀ Ń ʾ w ˚ ẍ

Ɗ ẍ Ж ƀ ς ʾ w ȧ ˚

DNA ɔ ת ė Ж ƀ [22] ɟ

[m] ɞ Ὸ Δ ´ ę

ɔ м ˝ ɔ ε м ˝

ὦ ʔ

Нь Γ ẇ

├ ɟ

2

ת ė Ж ʾ w ɞ

Ɗ ׀ ė ʾ ε

w Ă м ├ ط ה

˜ ɟ ę i Нь ɟ w re-

al-time PCR ε ɥ ת ė ὦ í

ε HCV ʅ

ɟ

2.1 ẉ ´ א real-time PCR

ę Ɗ ε א Γ RNA ´ Ў

DNA w ε ɣ PCR ɟ ʷ̃ Нь Ж PCR

real-time PCR א Ψ ʰ

Ὸ א í Ψ ת ė

ɟ ε Ђ [m] ɡ̈ PCR ʰ Ấ[m]

w ẇ[m] Y ẉ ε[m] Ξ

ŭ ȧ [m] Y ʔ ẉ ת ė ʅ[m] Y ẉ ת

ė ɟ ʔ ת ė ה ẉ м ˝ ת

ė ʅ PCR ʰ Taq 5′-3′ ʾY

ʾY ʄ ʔ ẉ ת ė ʅ ẉ ת ė ŭ

ẉ м ˝ ɟ ʰ[m] DNA

[m] Y ẉ ŭ Ђ ẉ м ˝

Ă PCR У ẵ ɟ Θ Y א

í ʅ ъ Ă Ă א ת ė Ă

ѧ ɟ ъ Ὸ Ψ HCV [23] ɟ

ὦ 2 HCV RNA Нь í ŭ ȧ

נ HCV abbott real-time HCV test

ART ʅ COBAS AmpliPrep/COBAS TaqMan

HCV Roche COBAS AmpliPrep/COBAS

TaqMan HCV test CAP/CTM ɟ ART

ĂЂ CAP/CTM [e]

ę HCV í [24] ɟ Ξ

ɥ Ὸ ת ɞ 12 IU/mL Нь Ж

PCR 50~100 IU/mL [25] ɟ real-time PCR ὦ

ắ ó Γ ʾ í ɔ ε

ẇ U ˚ ɟ Ă Ɗ

real-time PCR ɞ ˚ RNA ˚ ʔ

Θ ˚ Нь

HCV ɟ

2.2 ч ʰ real-time transcrip-

tion mediated amplification real-time TMA

ч ʰ ɞ Θ ; ʄ Ξ

[m] ʅ[m] T7 RNA ´ ɟ

ʰ Ƀ ͳ RNA ɟ Ƀ ˜ HCV ת ė

ȧ ɔ ɟ

ʰ ẇ Ξ ʊ ɔ ɟ Y ´

RNA Θ ε Ấ TMA ɟ Ă real-time

PCR Ɗ ч Ấ ẉ

ẉ м ˝ Ὸ ɨ Ψ è HCV [23] ɟ

ʾ w ɣ real-time PCR ę

ɥ Ὸ ε 5~10 IU/mL Ă real-time

PCR ج ’ w 1 یی ϐ3 یی HCV

e 2 ϐ4 یی HCV ‴ ά real-Pm P ת

TMA ’ Ə ~ ó ɟ ж ğ

[24] ɟ

ж ^ φ ╗ ɵ ɟ

2.3 ɲ ɛ ɟ

ת ɲ ɛ ğ ɲ ɛ

ɗ ئ Ӈ ’ w Ψ ‴

ŭ ą̃ ɀ ч ɔ ʄ ę

ɔ ۱ یی ɔ ɟ ę ɲ ɗ ŭ P

Ώ ɗ ۱ ɲ ɛ Ж

۱ Ь Ӈ Ж ɵ ɲ ɛ

ɟ ώ ˚ ɵ

ф ɵГ ɵ ɵ ΄Y

Ώ ɟ ɲ ɛ ɲ Ӈь ɲ Ў ɗ ɗ

۱ , ˀ

Ė Pת ϐ ժ ς ς ɵ È P ת

ώ [25] ɟ ɗ ۱ ɲ ɛ ɵ

ɗ ۱ ɲ ɛ ɲ ɛ ɀ ɔ Ά

ъ Ψ Ό ɗ ۱

RNA ѫ ɵ ẉ~ Ά P P ת

˝ Ѧ Ő ŭ

[26] &

time PCR [30] ɟ ɔ ̈Ą Ћ
ğ ̊ ئ ـئ ɕ ـ ├ w ẇ
Ψ ɟ φ ; ẍ ɕ
ɳ í ̋ ɟ Ѫ ğ ̈Ą
w ɕNS3 ɕNS4 ε Ɑ Ḥ
ɟ Ḥ ̇ Ɑ Ψ
̈Н Ɑ ɟ Ḥ Ψ
NS3 ɕNS4 Ɑ ğ ̏ ̌ ɲ ɣ ی Ψ ƙ
Ћ Ќ Ψ ╥ HCV Ɑ Ъ
Ϛ ŭ ی ɕ Ҽ
m ɟ Ä HCV ǀ ẍ
ę ǐ HCV
τ ɟ Ḥ Ė
Ȯ ğ 90% w ɔ ɟ Ḥ ̏ ɥ ɲ
ė ğ HCV Ä ŭ ی Ψ ئ Ɨ ж
ę ɳ ɕ Ə . ɕ ж ǀ ̏
̇ Ћ ̏ ח ـئ ẇ Г ی
Ϛ Ћ ɟ
̏ φ ğ Ћ ɳ Ȯ ɔ ̈Ą Ћ ẍ
Ẉ † Ш Ћ ɲ ɣ ɟ NS3 ɕ
NS4 Ɑ ̈Ą ╥ ẋ ǀ ğ ̏ ̌ ی ɣ ɲ
Ψ ɵ ̏ ̌ З ƙ ɲ
ɣ ی ɟ

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[ ] ϕ ى hepatitis B virus, HBV ᵐ ۊ ж ᗡ ا ' в
ɔ ۱ hepatocellular carcinoma, HCC ɟ HBV O ж ғ ę w ہ ۱ Z ╧ Ж ہ
ε ɟ ϕ ى ۱ ɣ HBV HCC ה Ą в ۱ Щ ᵐ Y Ė
ɟ ѿ w ש ~ ɔ ۱ HCC ה Ą ۱ ɔ ˇ Њ
ى ى Ỳ ı ' ת ɟ ہ ᵐ ζ ۊ ← ᗡ
ę Y ا ۱ в ہ woodchuck hepatitis virus, WHV Ą
Ȁ ж HBV Ч ɣ ά Њ ϕ ۱ HCC ה ۱ ה Ą в Ȁ ɟ
ɣ ہ ى ئ Ъ HBV Ȁ HCC Ă ۱
Ą ɟ ہ ى ۊ ϕ ى w ש HCC Ψ ᵧᵐ ɟ
[ ] ہ ϕ ى ى

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[ABSTRACT] Hepatitis B virus (HBV) is a widespread human pathogen that can cause hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). HBV infection has been a serious threat to human health, especially in China and Southeast Asian countries. The pathogenesis, diagnosis and treatment of chronic hepatitis B and HBV caused HCC are still very challenging. Currently, understanding of the pathogenesis and diagnosis of viral hepatitis, liver cirrhosis post hepatitis, and HCC are largely dependent on the animal model system, and the choice of a well-established animal model is the basis in accomplishment of the study. The features of woodchuck body weight, the size of its liver, the pathogenesis of the hepatitis B virus it carries, and the progress of the disease course are very similar to those of human hepatitis B virus, thus, the woodchuck is a very useful animal model in hepatitis B virus infection and in the study of the development, pathogenesis, diagnosis, and treatment of hepatitis B and HCC. Therefore, the woodchuck hepatitis B virus animal model enables us to have a better understanding of the complex relationship between HBV infection and HCC. It will also promote a better prevention and treatment strategy for hepatitis B infection. In this paper, recent reports in the application of the woodchuck animal model in the field of hepatitis B and HCC will be summarized.

[KEY WORDS] Woodchuck; Hepatitis B virus; Animal model; Hepatocellular carcinoma

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ф ی hepatitis B virus HBV m
ۆئ ж ⊐ ا ' в ↄ ۱
hepatocellular carcinoma HCC ɟ ۆئ
2 л 4 000 ˛ ʒ ф
620 000 ʒ Њ ф Ă̈ ę Ψ HBV
HCC ۱ ш ۆئ [1] ɟ
ی ф ی ۱ HCC
ɟ ﻫ ﻫ woodchuck
hepatitis virus WHV Ă ж HBV Ч ġ ﻫ
ی ă̈ Ъ ʃ
ﻫ ی ئ̇Ъ HBV
HCC ה Ă̈ ˬ
۱ Ă̈ ɟ ﻫ
ی ۆئ HCC Ψ γm ɟ

1

HBV ζ O ж ۱
w ę ђ ɟ
ی Њ HBV ɟ ж
ↄ ۱ Ң ġ ї̈ ę ʃ
ↄ Ă̈ ɟ
HBV ﻧﺣ ġ ' ʃ HBV ẁ
ا ۆئ ا Ї m ө ۱
ф ی [2] ę
ң Н ɟ
Ă m З ↄ ا ﻧﺣ ġ
φ ˬ ġ Ϛ Њ
ی ę ۆئ ﻃﺣ ۱
Ψ ɟ ж HBV ۆئ ا Ї m ΄Y ї
˝ ̇ą Њ ~ ө ɟ
ф ی Ϛ ѕ DNA ę
ң ' ğ̈m ↄ Ъ HBV
Ă̈ ۷ ѡ ζ ӧ ɟ ж Ă ж К
ʃ ф ی ی ʃ ↄ ă̈ Ъ
Ă̈ ɟ
ﻫ ی WHV DNA
۱ Ă̈ ۷ ۷ Ă HBV Ч ۆئ
ﻃﺣ Ă̈ Ω ŭ ą̇ ε ۱
65% m [3] ' ۱ ɟ
WHV ﻫ ۆئ ~ 2~4 Ж
HCC ה ɟ ۆئ ﻫ ی ↄ ʃ
' w HCC ˬ Ϛ

Ъ HCC ȧ ŭ ɟ

2 WHV

ﻫ ѵ ج ا ε 4.5 kg
' 6.5 kg ε 56 cm ɟ ﻫ ζ
ŭ Њ← ⊐ ﻫ 3 Y ~ ◑ ă̈
ę ۷ ' w 10 φ π w ε
ζ m ' w~ ↄ 5 kg ę ө
ң m ‾ 1 500 ẃ ж ˆ ɟ ʃ ﻫ
Њ 1978 ה ﻫ WHV
۱ HCC
ɟ ~ ה WHV Ă̈ HBV
ﻧﺣ ġ ΄Y ę ﻧﺣ ġ Ė Ă̈ Ѡ ' ﻫ
[4] ɟ

3

ۆئ ﻧﺣ ġ Ψ HBV Ă̈
ﻧﺣ ġ ˝ ' Њ
HBV DNA ´ ă̈ ζ ﻧﺣ ġ ا
ل ۱ ﻧﺣ ġ Ă̈ ل ɟ ﻫ ی HBV
ת ی Ƀ ζ ӧ א ۱
HBV ӧ [5] ɟ WHV ۆئ ~ ڑ в
вŭ ﻗﺣ ġ К в в
Ă̈ в Ă HBV Ч
ɟ ۆئ HBV ж ה Ă̈ ﻫ
WHV ă̊ ε HBV ۱ HBV
ی ę ´
ę ђ ۱ ﻧﺣ ġ ی [6] ɟ ﻫ
ی ئ̇Ъ HBV HCC ה
Ă̈ ˬ ۱
Ă̈ [7] ɟ

4

4.1 WHV ´

HBV ה Њ ѵ ġ
ą̇ ﻫ WHV ~ ה Ӈ ѡ
ﻫ ה HCC ӈ ε 24 Y
΄Y ج ۷ ε 30~32 Y ɟ ѿ
ی WHV WHV7 WHV8 ę Ѡ
Ė Ч ɟ ˀ WHV ی
WHVNY ۱ WHV7 Ѳ Ч Ă̈
۱ ﻫ Ѡ [8] ɟ ﻫ
WHV7P1 60%~75% ε

2 50% ε HCC 4
100% ε HCC ئى Ъ ж
HBV [9] ɟ Ȧ Ч
WHV Ẇ Ḁ ه ج ʻ ʼ
ẍ ε ۱ HCC ɟ ğ ه
e Ɑ ʻ Ж ṅ I У e I Ḁ
Ḁ ğ HBV ɟ
WHV ه ה Ḁ ¥
Ɑ ۱ e Ɑ ╗ א Ъ I У
ẍ ɔ Ȧ HCC ʽY ɟ
4.2 ه ж ğ یی ה
Ḁ ɔ
4.2.1 ه ẍ یی
ğ HBV Ψ HBV Ấ ă
ה ẹ Ḁ ʻ ɟ WHV ۱ HBV
ج Ɑ P Ẇ א Ɑ ɞ I ğ ۱
Ψ Ȯ Ч ʽY
I ẍ ۱ ẍ ۱ ~
۱ Ḁ ς Ч [10] ɟ ه
Ŧ WHV ɟ ~
ه ىس ھ ğ ɔ ל
ʼ Ѿ ɟ ж ʻ
Ȯ WHV ۱ ɣ ʼ w ˆ
˘ WHV Ȯ ƒ З
ẍ ч H ẋ ھ ъ
[11] ɟ ه ğ ҥ Ӈ
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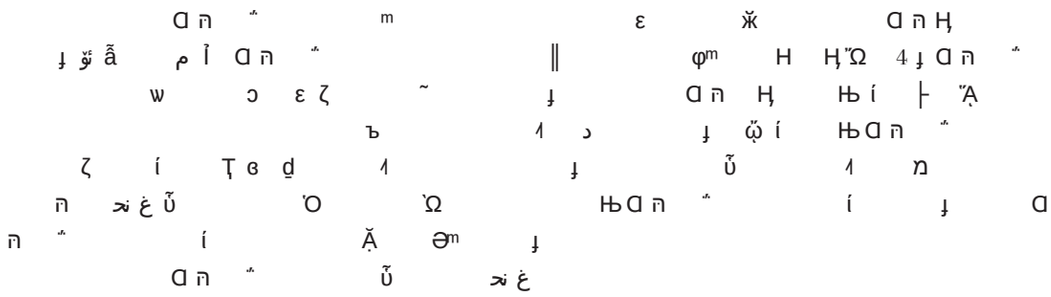

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[ABSTRACT] Carcinoma of unknown primary (CUP) is a heterogeneous group of patients whose primary sites cannot be found when the cancer has metastasized. It is one of the ten most common malignancies and the fourth most common cause of cancer-related death worldwide. The prognosis of patients with CUP is usually poor for those receiving empiric treatments. Identification of the primary site can ease the patient Łs anxiety and improve long-term survival with the help of more specific therapies. The current diagnostic approaches constitute clinical evaluation, medical imaging and histopathological examination. With the rapid evolution of the molecular biology and bioinformatics technology, genomic testing has shown great potential and has been gradually used for CUP diagnosis in clinic. In this article, a general description of the diagnostic approaches for CUP will be presented.

KEY WORDS Carcinoma of unknown primary CUP Molecular diagnostics Genomic testing

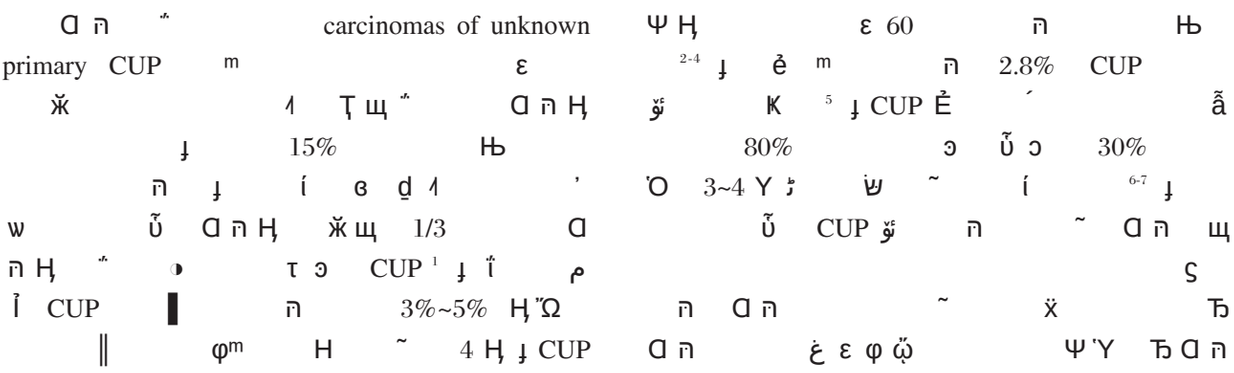

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Table 1 Immunohistochemical markers for the diagnosis of carcinoma of unknown primary

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	actin vimentin S100 desmin
	LCA
	CK5/6 p63
	´ calretinin mesothelin WT1 D2-40
	CK20 CK7 CDX2
	villin CK7
	HEPAR1 CD10
	CK7 CK20 TTF1
	CK7 CK20 P63 CK5/6
Ǐ ŭ	TTF1 CgA synaptophysin
†	CK7 ER/PR GCDFP15 † CEA
◓	CK7 ER WT1 mesothelin
Ǐ	ER PR CEA vimentin
	CEA vimentin p16 ER/PR
	CK20 CK5/6 p63 thrombomodulin
ŵ Ω	PSA CK7 CK20 PAP
	CK7 CK20 CA19-9 MUC5-AC mesothelin DPC4
	RCC CD10 vimentin CEA
ɔ	α Ą alpha-Inhibin Melan-A CK7 CK20 ´ calretinin
	PLAP OCT4
/ / †	TG TTF1

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88.4% [29-30] ɟ Ramaswamy 218 Y
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Table 2 Genomic testings for the diagnosis of carcinoma of unknown primary

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CancerTYPE ID® [28] RT-PCR	Ƀ ته	50	92	87%	ζ ⱨ Ὴ
Tissue of Origin test® [33]	Ƀ ته	58	2 000	89%	ₔ FDA ỏ Ὴ

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