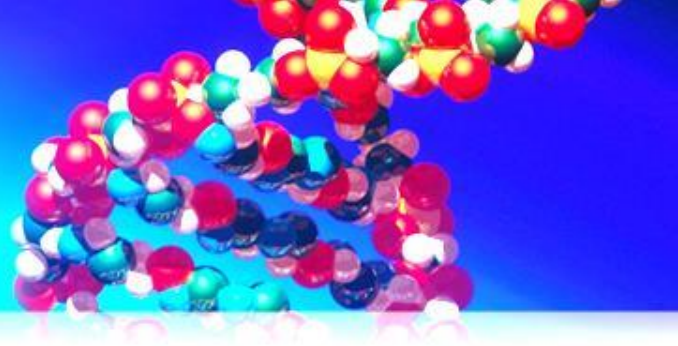
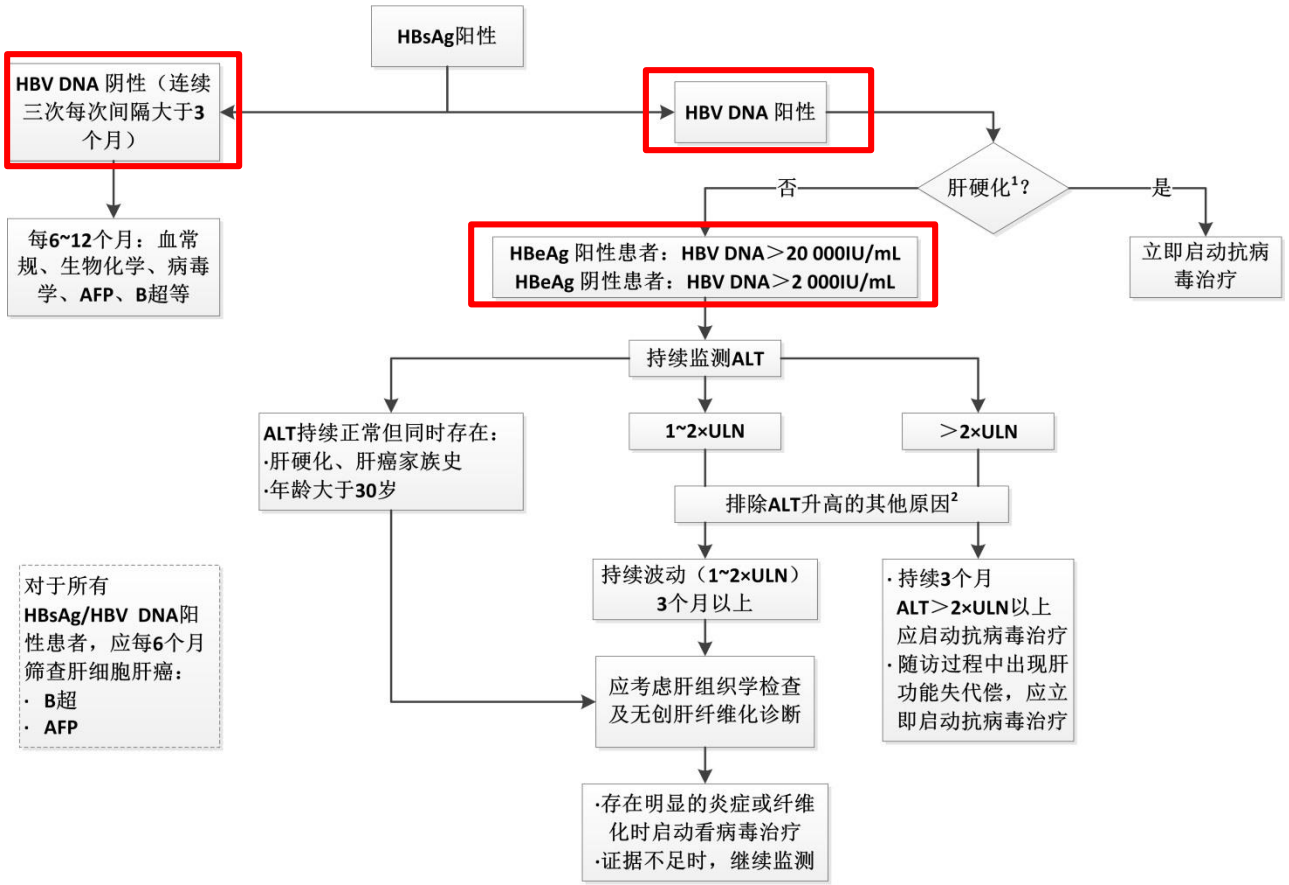


双重荧光PCR提高乙型肝炎病毒核酸检测性能的研究

临检中心　　王露楠

研究背景

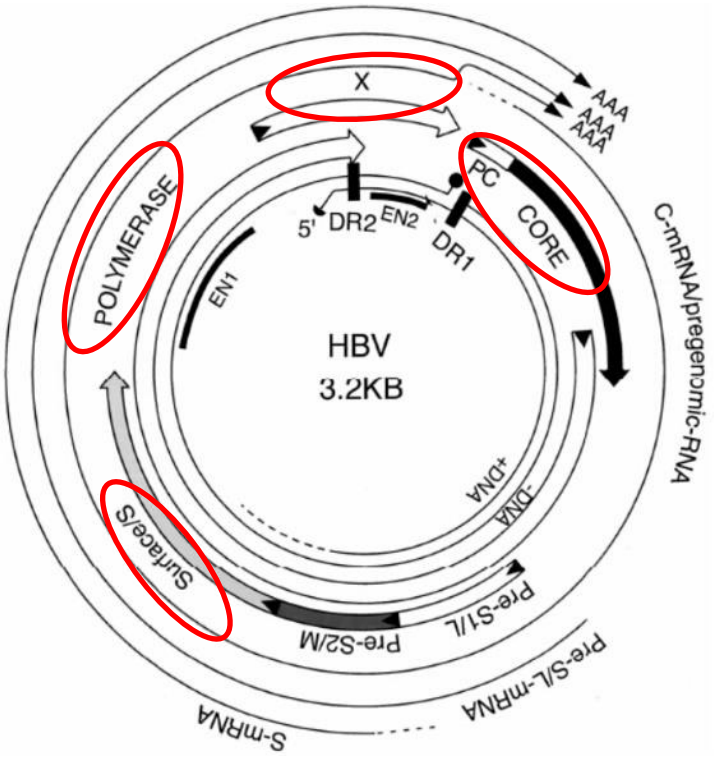
- 准确HBV DNA定量检测在乙肝抗病毒治疗过程中具有重要作用
- HBV DNA血液筛查可缩短HBV窗口期，是隐匿性乙肝感染（OBI）筛查的可靠指标。



1. 2015年《慢性乙肝防治指南》

研究背景

- HBV是一种部分双链环状的DNA病毒，全长约3200bp，根据编码蛋白质的不同可分为S、C、P和X区；
- 大多HBV DNA定量试剂和血液核酸筛查试剂扩增区域位于S或C区；



试剂	检测区域	定量下限	灵敏度
罗氏定量	C区	20 IU/mL	
达安定量	C区	100 IU/mL	
罗氏血筛	C区		2.3 IU/mL
浩源血筛	S区		6.3 IU/mL

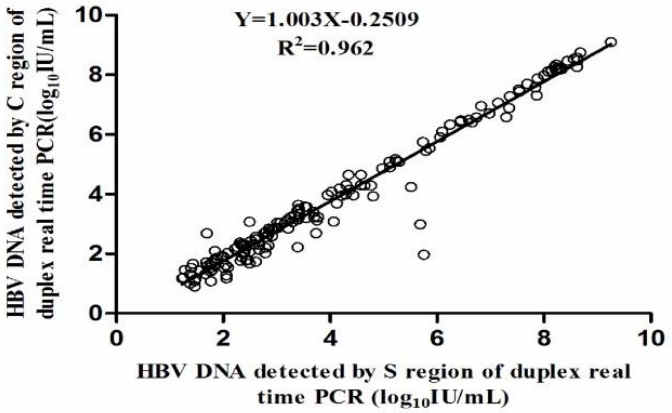
1. Alter MJ, Margolis HS. The emergence of hepatitis B as a sexually transmitted disease [J]. Med Clin North Am. 1990,74(6):1529-41

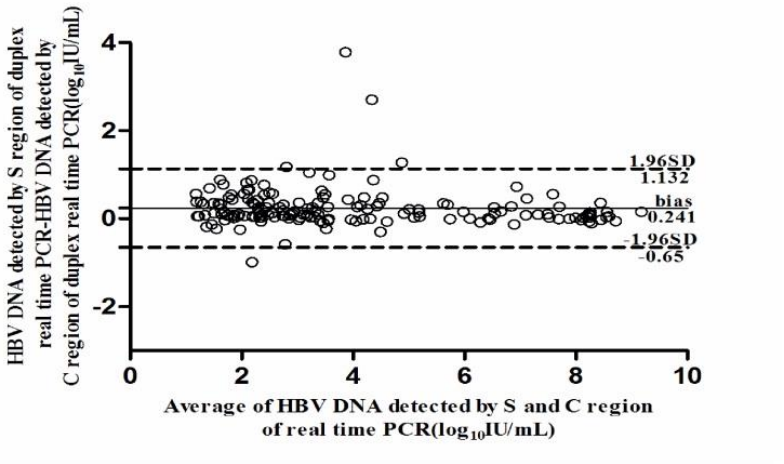
双重荧光定量PCR方法S区和C区定量值一致性分析

双重荧光定量PCR方法中S区和C区定量值相关性分析：

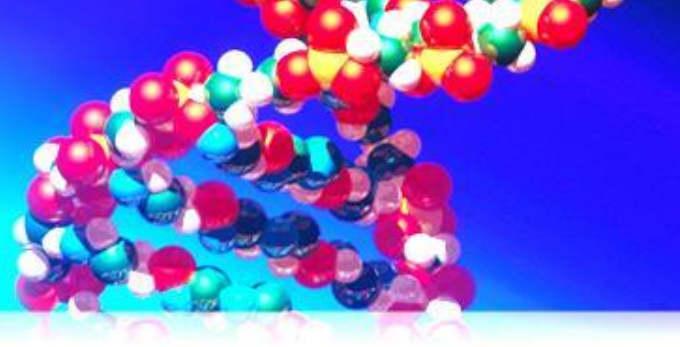
具有良好相关性，

R^2=0.962





双重荧光定量PCR方法S区和C区定量值一致性分析

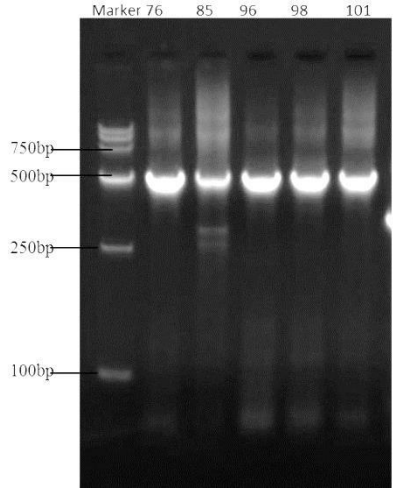


- 双重荧光定量PCR方法方法中S区和C区定量值差1.0log_{10}IU/mL的标本：

Sample No.	S区（logIU/mL）	C区（logIU/mL）
302-76	5.69	2.99
302-85	3.74	2.69
302-96	5.75	1.97
302-98	5.51	4.24
302-101	3.39	2.22

5例标本双重荧光定量PCR方法中S区定量值高于C区定量值1.0log_{10}IU/mL以上。

双重荧光定量PCR方法S区和C区定量值一致性分析



Forward primer (2328) Probe Reverse primer(2447)

TTCCGGAAACTACTGTTGTTAGACGAAGAGGCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCGCAGACGAAGGTCTCAATCGCCGCGTCGCAGAAGATCTCAATCTCGGGAATCTCAAT

-------G----------T----------A----------

----------A----------

----------C----------

----------C----------A----------

----------C---------C------

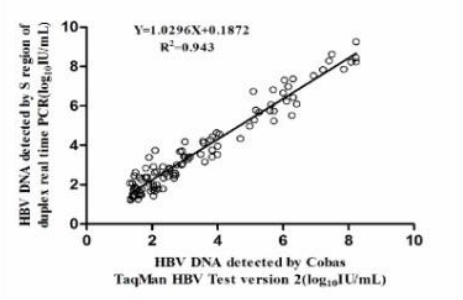
C区定量值偏低的标本其引物结合区存在突变

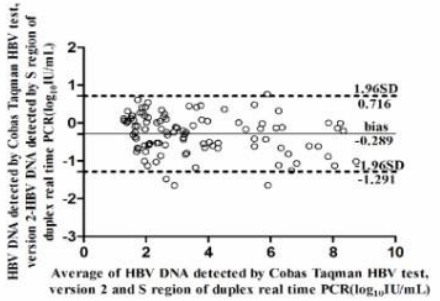
双重荧光定量PCR方法S区和C区定量值一致性分析

- 双重荧光定量PCR方法定量值与罗氏定量试剂103例HBV标本定量相关性分析 ：

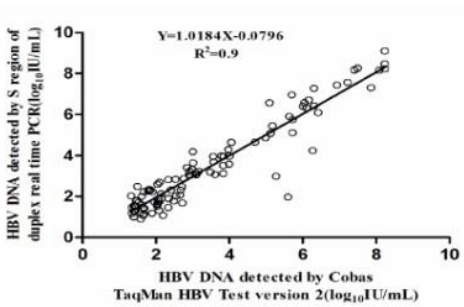
R^2=0. 944

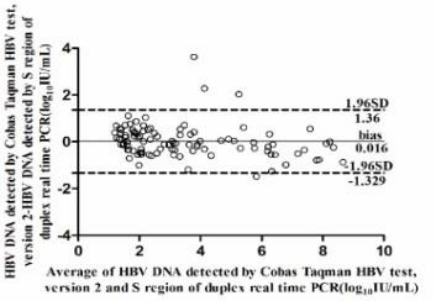
S



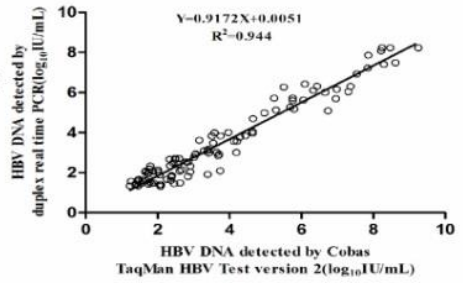


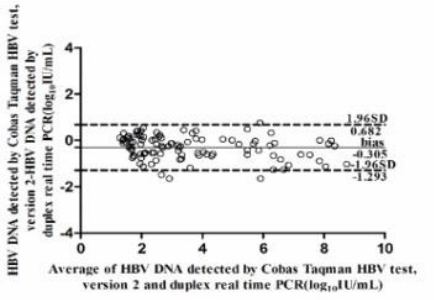
C





S+C





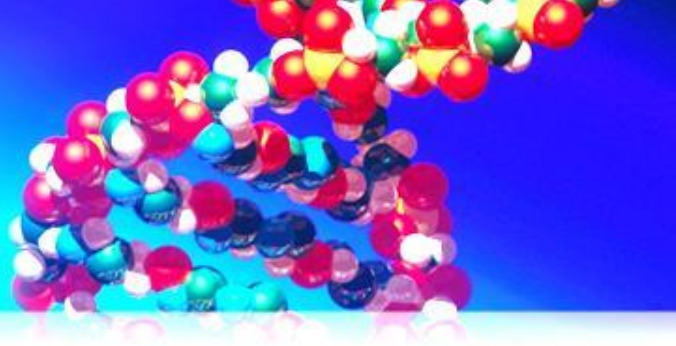
双重荧光定量PCR方法S区和C区定量值一致性分析

- 双重荧光定量PCR方法与罗氏定量试剂103例HBV标本定量值差1.0log_{10}IU/mL的标本：

Sample No.	罗氏（logIU/ml）	S区（logIU/ml）	C区（logIU/ml）
302-1	1.81	2.84	2.27
302-17	1.49	2.62	2.49
302-44	3.00	4.18	4.19
302-45	7.49	8.62	8.26
302-66	6.30	7.37	7.28
302-68	5.70	6.82	6.96
302-74	1.45	2.46	1.80
302-76	5.09	6.74	6.57
302-80	6.04	7.30	6.58
302-85	2.09	3.74	2.69
302-101	1.91	3.39	2.22

11例标本双重荧光定量PCR方法定量值高于罗氏定量试剂定量值1.0log_{10}IU/mL以上。

双重荧光定量PCR方法S区和C区定量值一致性分析



- 对定量不准确的标本扩增其全长或者pre-Core和Core区，选用4个双重qPCR与罗氏定量值一致的标本进行扩增和测序，作为对照序列与定量不一致的标本序列进行比对分析：

Sample. No.	Different sites
44	C1962T, T1963G
45	T1858C, G1915C, T1936C
66	G1915C,
76	G1899A, G1915A, G1937A, T1938A, T1961G, C1962G

T1961G, C1962G/T以及T1963G位点突变与已报道加拿大人群突变位点一致

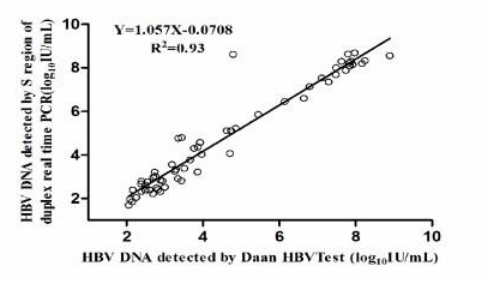
Andonov A, et al. Sequence variability of the Cobas taqman assay target region impacts accurate HBV DNA detection. Vox Sang. 2016;111:S58.

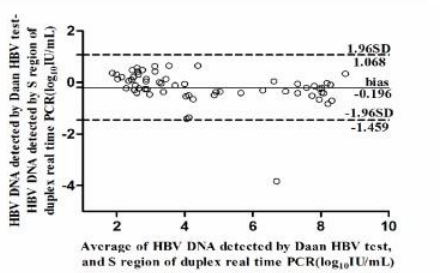
双重荧光定量PCR方法S区和C区定量值一致性分析

双重荧光定量PCR方法与达安定量试剂盒对59例HBV标本定量相关性分析：

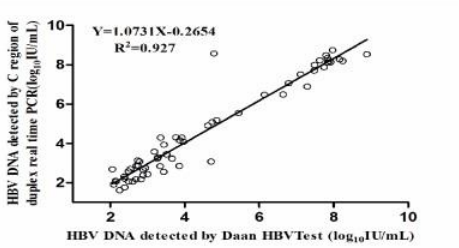
R^2=0. 93

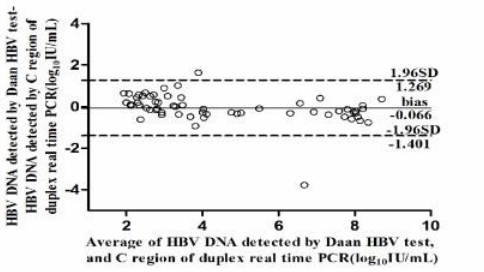
S



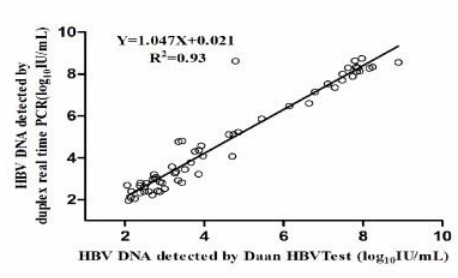


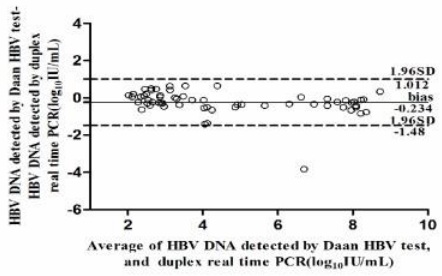
C



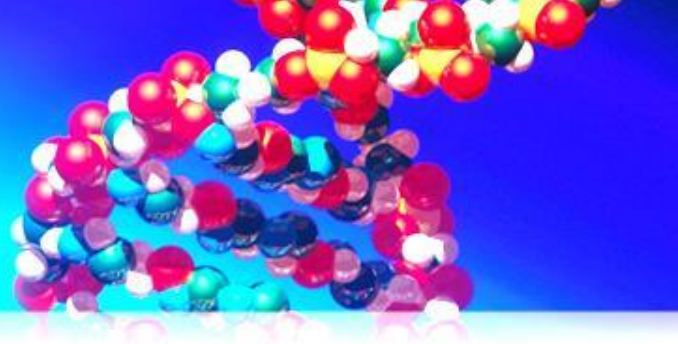


S+C





双重荧光PCR方法在血筛检测中的结果分析

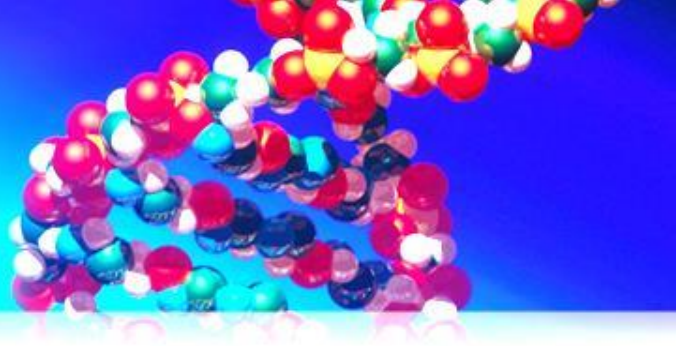


S和C区Ct值差别明显的标本：

编号	S区Ct	C区Ct	浩源血筛Ct	罗氏血筛Ct	雅培定量（IU/m）	罗氏定量（IU/m）
1	30.2	38.14	25.6	26.1	2510	4200
2	34.32	37.65	29.9	29.7	357	263
3	36.25	40.94	31.35	34.4	87	60.4
4	36.7	41.24	30.97	33.2	153	125
5	46.54	36.79	31.68	33.5	91	116

4例子标本C区Ct值落后S区3个罗氏Ct以上，1例标本S区Ct值落后C区近10个Ct

双重荧光PCR方法在血筛检测中的结果分析



S区序列分析：

Forward Primer（nt376）　　Probe　　Reverse Primer（nt467）

GATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTACCAAGGTATGTTGC

1 ----------

5 ----------G----------

C区序列分析：

Forward Primer(nt2328)　　Probe　　Reverse primer(nt2447)

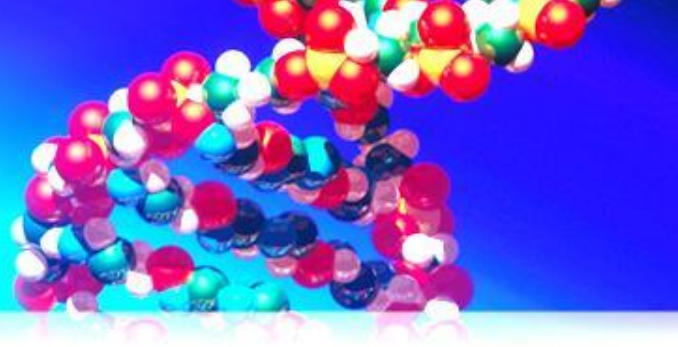
TTCCGGAAACTACTGTTGTTAGACGAAGAGGCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCGCAGACGAAGGTCTCAATCGCCGCGTCGCAGAAGATCTCAATCTCGGGAATCTCAAT

1 ----------A----------

5 ----------

经测序，两例标本各自在S和C区引物结合区存在错配。

双重荧光PCR方法在血筛检测中的结果分析



对浩源和罗氏血筛试剂单试剂和双试剂阳性标本定性筛查方法检测：

浩源血筛检测	罗氏血筛检测	S区阳性/总体检测数	C区阳性/总体检测数	S+C阳性/总体检测数
+	+	22/22	22/22	22/22 (100%)
+	—	10/25	11/25	14/25 (56%)
—	+	16/21	19/21	19/21(90.5%)

说明单区段的商品血筛试剂存在因标本浓度过低或者区段错配而导致的漏检。

双重荧光PCR方法在血筛检测中的结果分析

样本编号	浩源单检（Ct）	罗氏单检（Ct）	雅培M2000定量值（IU/mL）	罗氏（IU/mL）	S区-Ct值	C区-Ct值
6LX07001	25.65	26.1	2510	4200	30.20	38.14
5SY05001	29.99	29.7	357	263	34.32	37.65
6DQ06001	29.28	30.8	313	184	34.33	33.41
6QD06002	31.39	32.7	250	66	34.87	37.33
5SY04007	31.36	34.4	87	60	36.25	40.94
5XY03001	30.97	33.2	153	125	36.70	41.24
6YC06001	31.30	33.5	82	33	37.21	36.72
5SY04006	32.08	33.8	54	—	38.51	37.42
5HA12002	Undet	35.2	54	＜20	38.84	37.84
5SR11001	Undet	34.5	27	＜20	39.62	37.67
6CD02003	Undet	36	＜10	＜20	40.88	39
5QN05002	35.35	Undet	—	—	41.03	40.89
5SR11005	Undet	36.4	＜10	—	41.08	39.49
6SX04006	Undet	37.8	12	—	42	39.05
5SY04002	36.26	Undet	14	—	42.67	46.15
6XZ05002	Undet	29.8	139	97	**36.65**	**35.4**
6SX04007	Undet	36.3	＜10	—	45.71	39.5
6YC04003	31.69	33.5	91	116	46.54	36.79
6SX03015	Undet	35.9	＜10	＜20	—	40.22
6QD06005	35.71	Undet	—	＜20	—	41.75
5SY05005	35.47	Undet	—	—	—	—

感谢聆听！