



Food and Agriculture
Organization of the
United Nations



Proposed FMD Molecular Diagnostic Algorithm

Results and REG's next steps

1st Regional Expert Group Meeting on Foot and Mouth Disease

14 – 16 May 2019, Bangkok Thailand

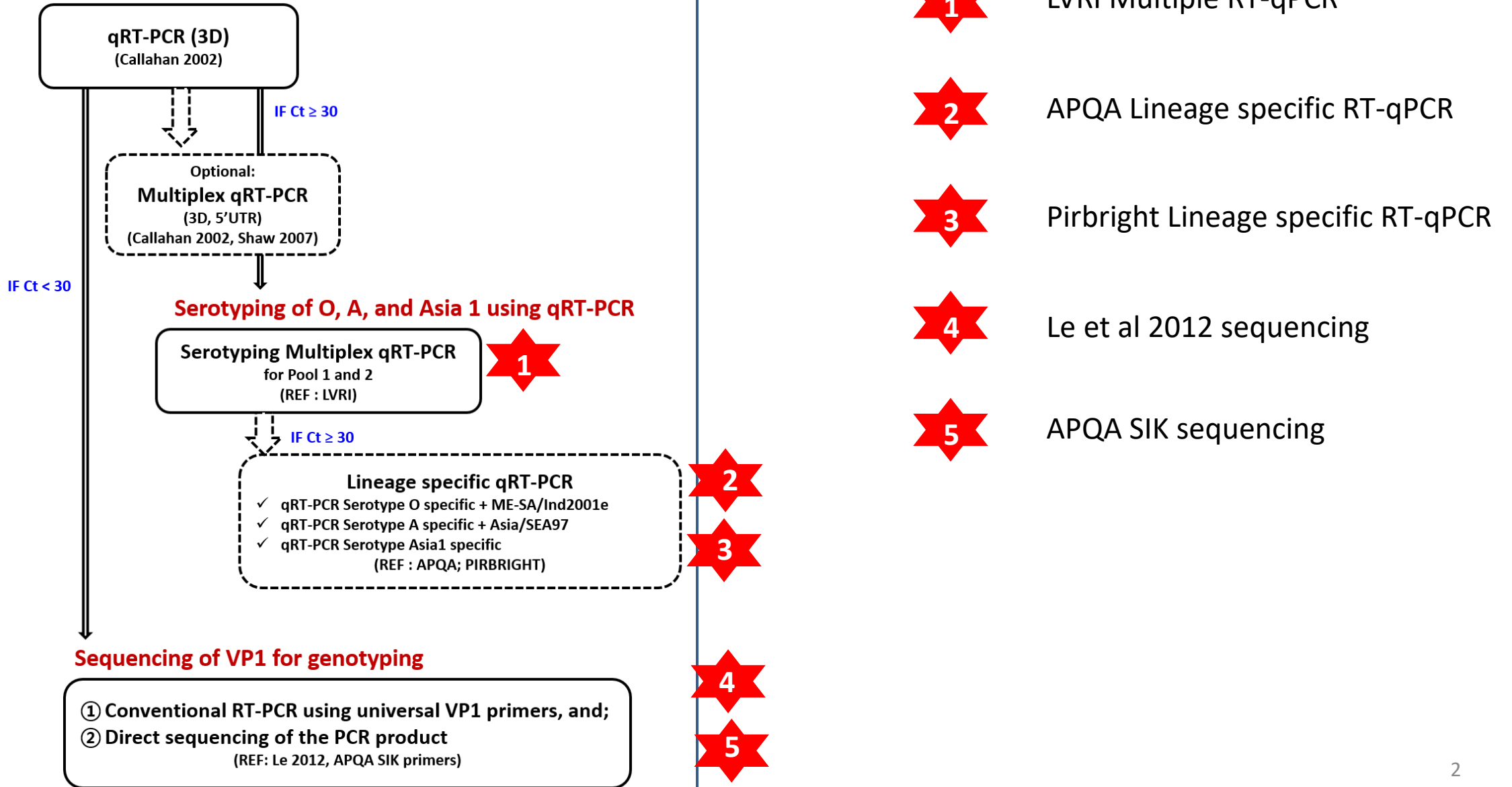


中华人民共和国农业农村部
Ministry of Agriculture and Rural Affairs of the People's Republic of China



Draft FMD Molecular Diagnostic Algorithm

Pan-serotype detection of FMD



Progress in three OIE reference laboratories on FMD

	LVRI serotyping RT-qPCR (kits)	APQA lineage-specific RT-qPCR (kits)	Pirbright lineage specific RT-qPCR	Universal primer for VP1 sequencing	APQA SIK primers and protocols for VP1 sequencing
APQA	Y	Y	Y	Y	Y
LVRI	Not Available	Y	Y	Y	Y
RRL-Pakchong	Y	Y	Y	Y	Y

RRL Pakchong

	Protocol	No. of Samples	No. Amplified	No. Sequenced	O	A	Asia 1	O Panasia	O Mya98	O Ind 2001	O Cathay	A SEA-97	A G-VII	Asia 1
4	VP1 sequencing using Universal primers (Le, 2012)	12	10	7	2/7	3/3	2/2	2	-	-	-	3	-	2
5	VP1 sequencing using SIK primers (APQA)	12	8	7	3/7	2/3	2/2	1	1	1	-	2	-	2
								Primers	Primers	Primers	Primers	Primers	Primers	Primers
1	LVRI Serotyping O/A/Asia1 rRT-PCR	12	12		7/7 <u>1/5</u>	3/3	2/2	-	-	-	-	-	-	-
2	APQA Lineage specific rRT-PCR	12	12		7/7 <u>0/5</u>	3/3	2/2	-	-	2/2	-	3/3	-	2/2

Black: positive/tested

Red: cross reactions

LVRI

	Protocol	No. of Samples	No. Amplified	No. Sequenced	O	A	Asia 1	O Panasia	O Mya98	O Ind 2001	O Cathay	A SEA-97	A G-VII	Asia 1
4	VP1 sequencing using Universal primers (Le, 2012)	13	2					0/2	0/4	1/2	0/2	1/2	0/0	0/1
5	VP1 sequencing using SIK primers (APQA)	13	9					1/2	3/4	2/2	1/2	2/2	0/0	0/1
								Primers	Primers	Primers	Primers	Primers	Primers	Primers
1	LVRI Serotyping O/A/Asia1 rRT-PCR													
2	APQA Lineage specific rRT-PCR	23			18	4	1	3	3	3	1/2	3	0	1

Black: positive/tested

Red: cross reactions

APQA

	Protocol	No. of samples	No. Amplified/ Sequenced	O	A	Asia 1	O Panasia	O Mya98	O Ind 2001	O Cathay	A SEA-97	A G-VII	Asia 1
4	VP1 sequencing using Universal primers (Le, 2012)	29	9	4/16	1/9	4/4	2/5	0/3	1/5	0/1	0/4	1/2	4/4
5	VP1 sequencing using SIK primers (APQA)	29	25	15/16	8/9	2/4	5/5	2/3	5/5	1/1	4/4	1/2	2/4
							Primers	Primers	Primers	Primers	Primers	Primers	Primers
1	LVRI Serotyping O/A/Asia1 rRT-PCR	29		16/16 <u>1/13</u>	5/9 <u>0/20</u>	2/4 <u>0/25</u>	-	-	-	-	-	-	-
2	APQA Lineage specific rRT-PCR	29		15/16 <u>0/13</u>	9/9 <u>1/20</u>	4/4 <u>0/25</u>	0/5	0/3	5/5 <u>0/11</u>	0/1	4/4 <u>0/5</u> • <u>0(1)</u>	0/2	4/4

Black: positive/tested

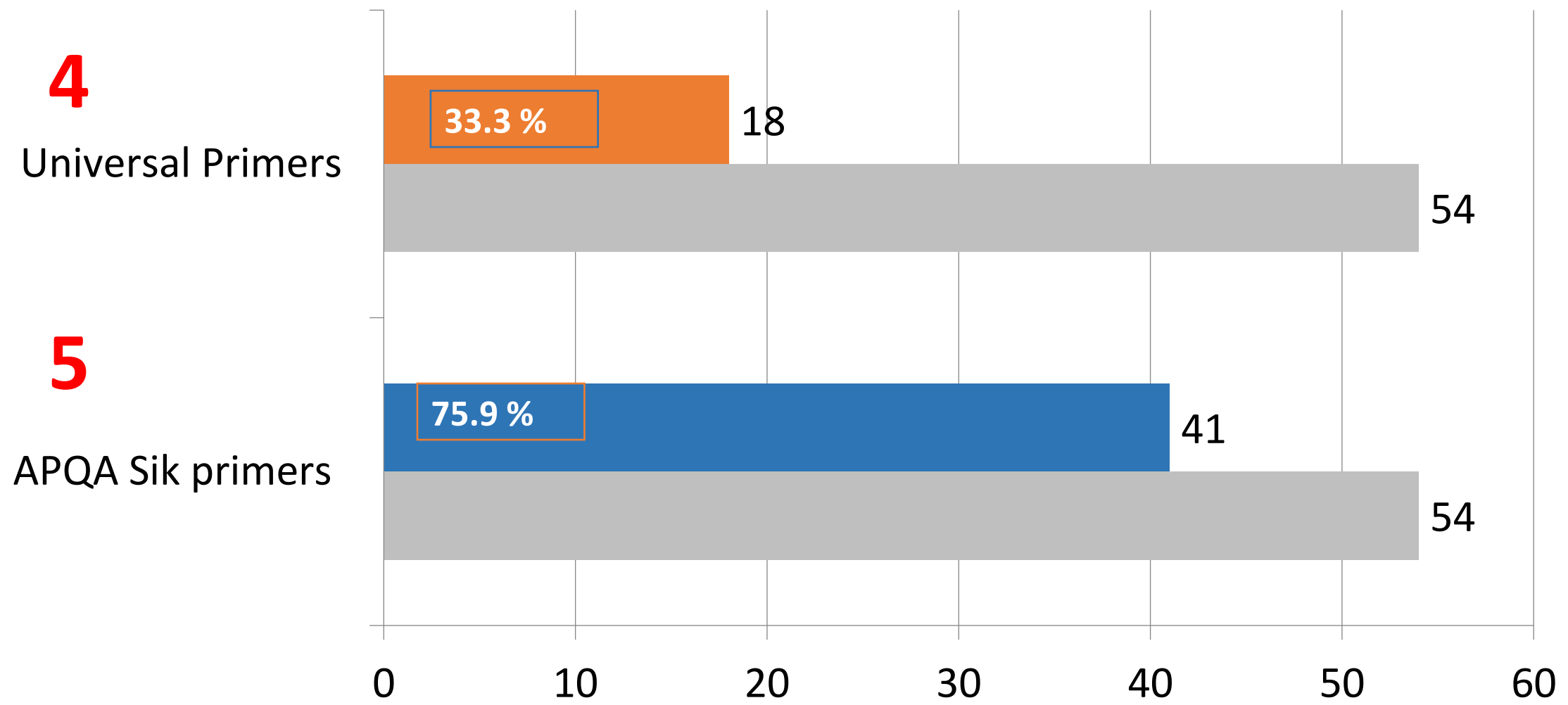
Red: cross reactions

Compiled results

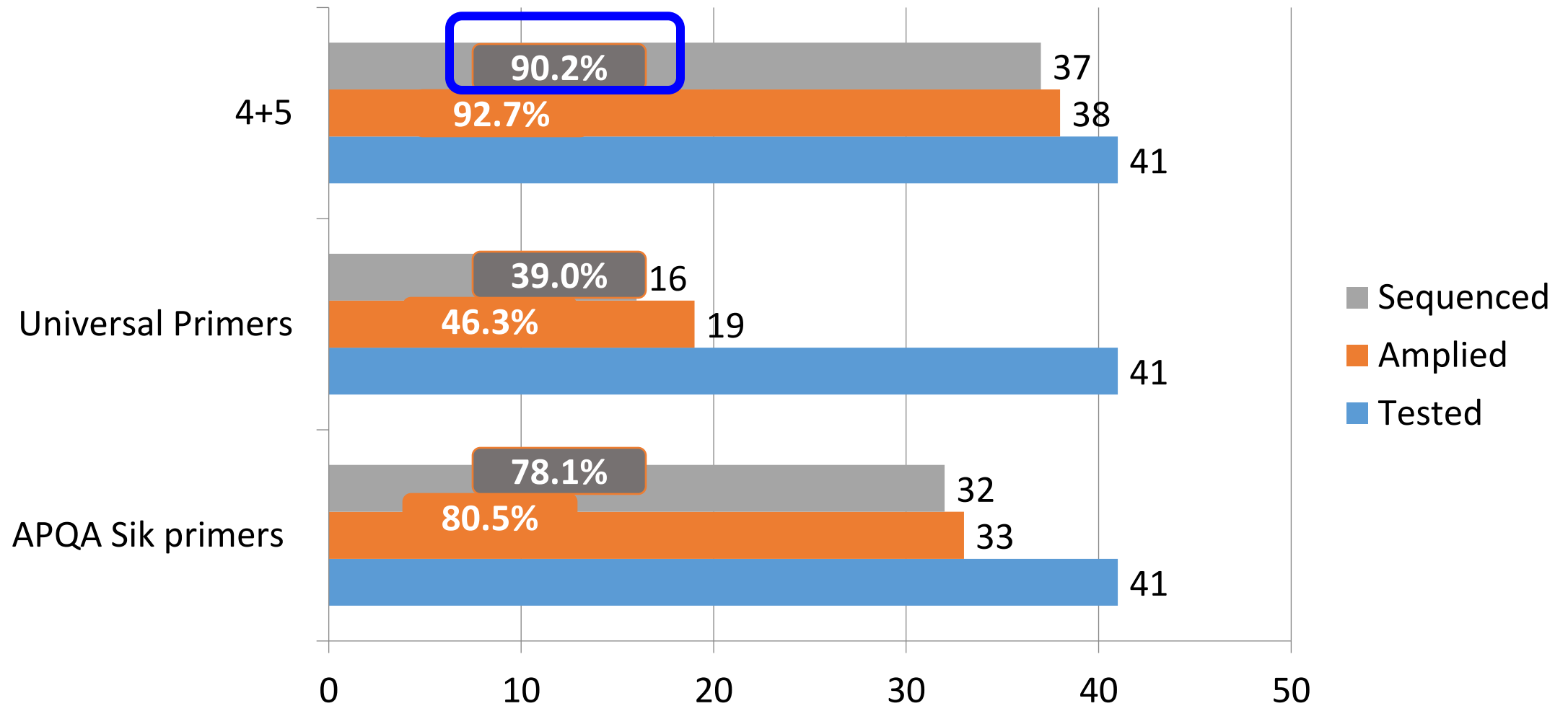
Sequencing VP1 for genotyping - results

Method	LAB	No. of samples	No. amplified	No. sequenced	O		A		Asia 1	
					POS	Tested	POS	Tested	POS	Tested
4 VP1 Universal primers (Le, 2012)	RRL	12	10	7	2	7	3	3	2	2
	LVRI	13	2	2	1	10	1	2	0	1
	APQA	29	9	9	4	16	1	9	4	4
	Total	54	18		8	33	5	14	6	7
5 VP1 SIK primers (APQA)	RRL	12	8	7	3	7	2	3	2	2
	LVRI	13	9	9	7	10	2	2	0	1
	APQA	29	25	25	15	16	8	9	2	4
	Total	54	41		25	33	12	14	4	7

Sequencing VP1 for genotyping – results



4+5 Sequencing VP1 for genotyping – results

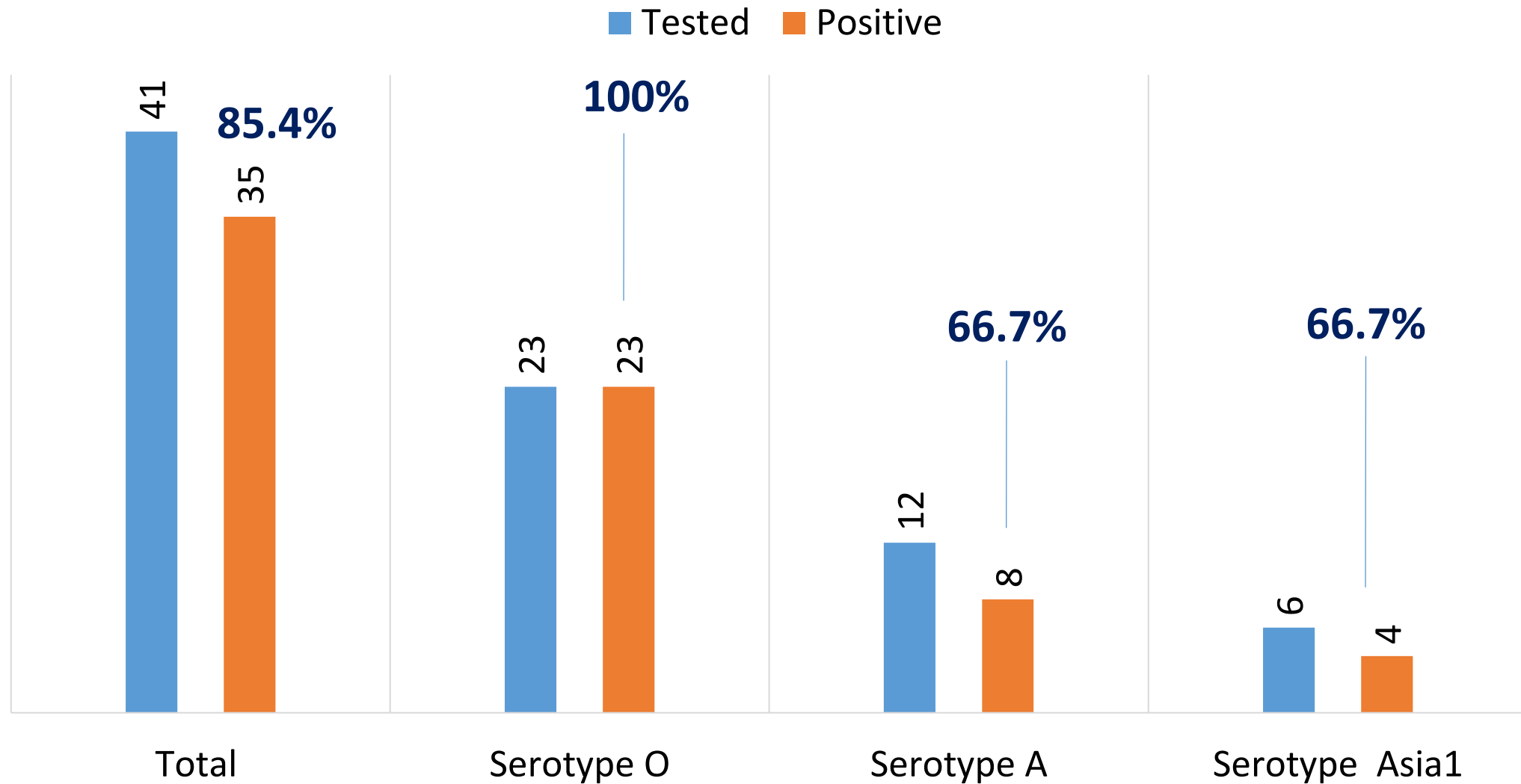


* Data from RRL-Pakchong and APQA

1 Serotyping (O.A.Asia1) Multiplex rRT-PCR

Lab	No. of samples	No. amplified	O		A		Asia 1		
			POS	Tested	POS	Tested	POS	Tested	
RRL	12	12	7 <u>1</u>	7 <u>5</u>	3	3	2	2	Cross reactions in primer for serotype O with Serotype A virus (1)
APQA	29	23	16 <u>1</u>	16 <u>13</u>	5 <u>0</u>	9 <u>20</u>	2 <u>0</u>	4 <u>25</u>	
Total	41	35	23	23	8	12	4	6	

1 Serotyping (O.A.Asia1) Multiplex rRT-PCR

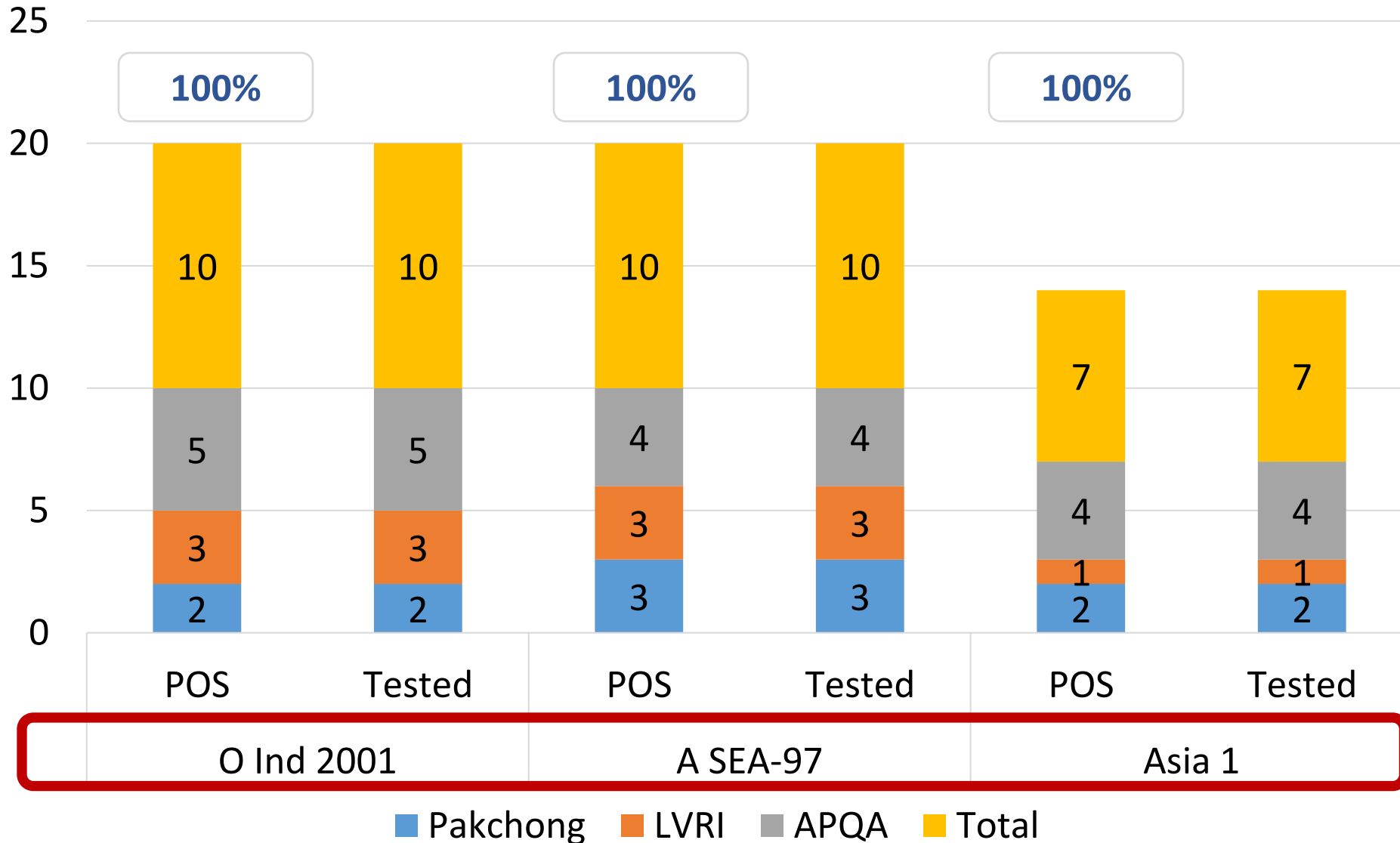


2 Serotype/lineage specific qRT-PCR (APQA)

Protocol	No. of Samples	No. Amplified	No. Sequenced	O	A	Asia 1	O Panasia	O Mya98	O Ind 2001	O Cathay	A SEA-97	A G-VII	Asia 1
							Primers	Primers	Primers	Primers	Primers	Primers	Primers
RRL-Pakchong	12	12		7/7 <u>0/5</u>	3/3 <u>0/9</u>	2/2 <u>0/10</u>	-	-	2/2	-	3/3	-	2/2
LVRI	23			18	4	1	3	3	3	1/2	3	0	1
APQA	29			15/16 <u>0/13</u>	9/9 <u>1/20</u>	4/4 <u>0/25</u>	0/5	0/3	5/5 <u>0/11</u>	0/1	4/4 <u>0/5</u> •O(1)	0/2	4/4

Cross reactions in primer for A/SEA-97 with Serotype O (1)

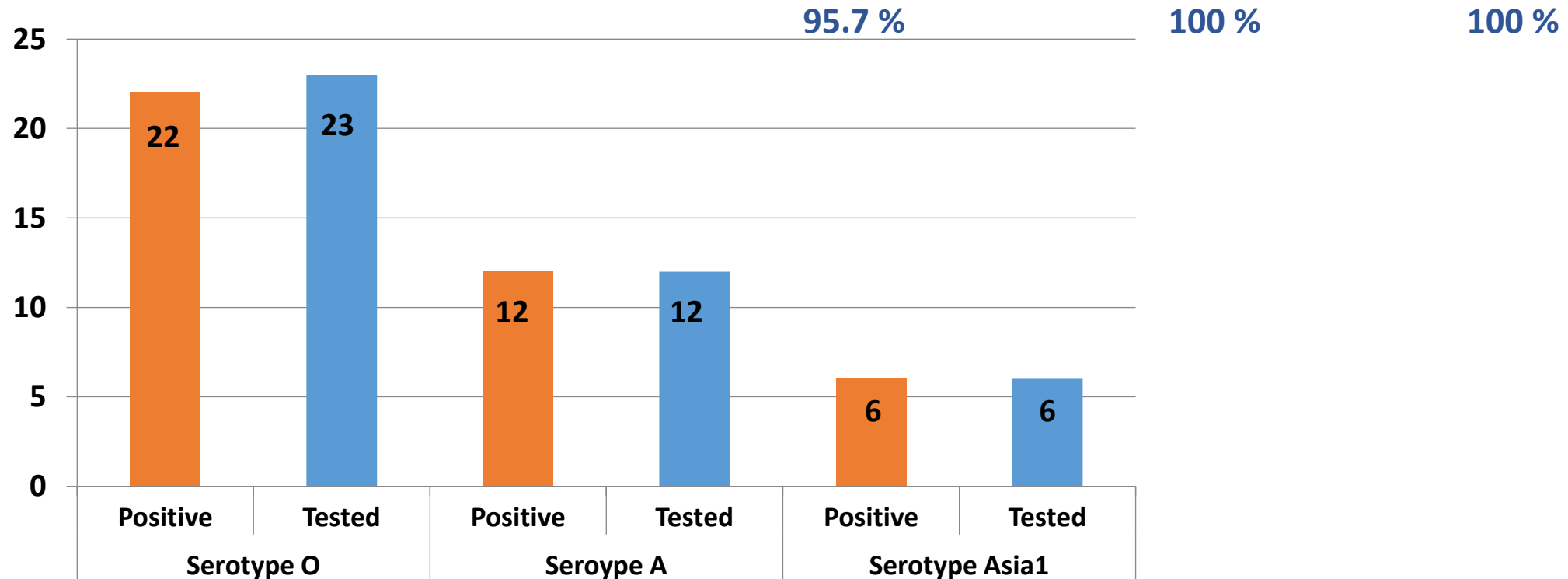
2. Lineage specific qRT-PCR (REF: APQA) - results



2. Serotype/lineage specific qRT-PCR (REF: APQA)

Serotyping O, A, Asia1

Institute	Serotype O		Seroype A		Serotype Asia1	
	Positive	Tested	Positive	Tested	Positive	Tested
RRL-Pakchong	7	7	3	3	2	2
APQA	15	16	9	9	4	4
Total	22	23	12	12	6	6



Proposed FMD Molecular Diagnostic Algorithm

Pan-serotype detection of FMD

qRT-PCR (3D)
(Callahan 2002)

IF Ct \geq 30

Optional:
Multiplex qRT-PCR
(3D, 5'UTR)
(Callahan 2002, Shaw 2007)

IF Ct $<$ 30

Serotyping qRT-PCR (O, A, and Asia 1)

Serotyping Multiplex qRT-PCR
for Pool 1 and 2
(REF : LVRI)

IF Ct \geq 30

Lineage specific qRT-PCR

Lineage specific qRT-PCR

- ✓ qRT-PCR Serotype O specific + ME-SA/Ind2001e
- ✓ qRT-PCR Serotype A specific + Asia/SEA97
- ✓ qRT-PCR Serotype Asia1 specific

(REF : APQA)

Sequencing of VP1 for genotyping

- ① Conventional RT-PCR using universal VP1 primers, and;
- ② Direct sequencing of the PCR product
(APQA SIK primers + Universal primers)

Further study or next steps

- I. **Piloting revised algorithm with more viruses (> 30 viruses in each laboratory)**
- II. **Inviting country FMD laboratories for piloting (Myanmar, Viet Nam)**

Acknowledgements

- OIE-RRL Pakchong
- OIE-RRL Lanzhou
- APQA
- The Pirbright Institute
- CSIRO-AAHL