Activities of ACDP in Asia-Pacific

Nagendrakumar Singanallur Balasubramanian

Senior Research Scientist

Immunomics, Health and Biosecurity

Australian Centre for Disease Preparedness - CSIRO

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- Wilna Vosloo, DSR-ACDP
- Phoebe Readford and Gemma Clarke, ACDP-International Program

- SRRWOAH and SEACFMD-Mongolia Campaign

- FAO-WOAH Regional Expert Group for FMD in Southeast Asia

- WOAH Reference Laboratories – Pakchong and Lanzhou
- APQA – South Korea
- The Pirbright Institute

ACDP-IP Regional Footprint 2024

Achievements: Regional Emerging Disease Support [REDS]

- DAFF funded project to strengthen technical capability in FMD and LSD
- Assisting lead Indonesian laboratories to develop external quality assurance(EQA) programs for the Indonesian veterinary laboratory network
- PUSVETMA for FMD
- DIC Wates for LSD
- EQA consisting of network quality controls and proficiency testing programs for serology and PCR

Achievements: BICOLLAB

- CSIRO ACDP supports the delivery of a targeted laboratory capacity-building project in Indonesia: BICOLLAB
- This project is funded by DFAT's Global Health Division (GHD).
- One of the subprojects will evaluate the performance characteristics of LFDs for FMD detection in field conditions.
- Sub-project objectives
    - Identify LFDs suitable for antigen detection in FMD outbreaks
    - Evaluate the LFDs for their performance characteristics
    - Standardize methods to recover the FMDV genome from LFDs

Achievements: BICOLLAB

- Another subproject has supported the capacity-building activities in Pakchong.
  - Two scientists were trained in laboratory activities for FMD detection and characterisation.
    - Training on molecular diagnosis of FMD using real-time RT-PCR.
    - Training on Sanger DNA sequencing for FMDV 5'NTR and VP1 region of the genome to allow for molecular serotyping and classification

- Two scientists were trained in planning for a proficiency testing scheme with the following objectives
  - To improve the trainees' understanding of being an accredited Proficiency Testing Provider (PTP).
  - Overview of the requirements for PTP under ISO 17043.
  - The training focused on sample preparation and preparing quality-assured PT samples using homogeneity and stability under accreditation.
  - The secondary aim was to highlight the required documentation to be developed to meet international standard ISO 17043. Quality Assurance was not covered in this training.

- The four scientists spent 2 weeks in ACDP.

Achievements: LabCap PNG

- Laboratory capacity building project in Papua New Guinea
- Funded by the Australian High Commission in Port Moresby
- Strengthening laboratory and field diagnostic capability for priority diseases, including FMD.
- For the detection of FMD, we have implemented:
  - Real time PCR testing
  - Lateral flow devices
  - ELISA testing (set-up in progress)

NAQIA/DAFF/ACDP - FMD and LSD Preparedness Workshop, Lae, Papua New Guinea, 5-8 June 2023

Pakchong FMD Laboratory- Field mission planning

- One ACDP staff joined two experts from WRLFMD on a field mission to Pakchong FMD Laboratory with the following objectives.
- Identify technical support and training needs for Pakchong FMD Laboratory
- Evaluate current diagnostic capacity for FMD diagnosis, including:
  - Validate via inter-laboratory comparison that specific reagents produced by Pakchong are fit for purpose.
  - Examine the proficiency tests for various methods, including serology, PCR and serotyping.
  - Evaluate the reagents and test kits produced by the Pakchong laboratory and all test methodologies used.
  - Examine assay validation reports and their fitness for purpose (cut-offs change depending on purpose)
  - Schedule to exchange reagents for validation and proficiency scheme if needed.

FMD vaccine matching studies

- Nagendra Singanallur from ACDP visited Pakchong FMD lab to perform the FMD vaccine matching studies with serotype A viruses.

Food and Agriculture Organization of the United Nations

World Organisation for Animal Health
Founded as OIE

Outputs of FAO-WOAH FMD REG meetings and activities

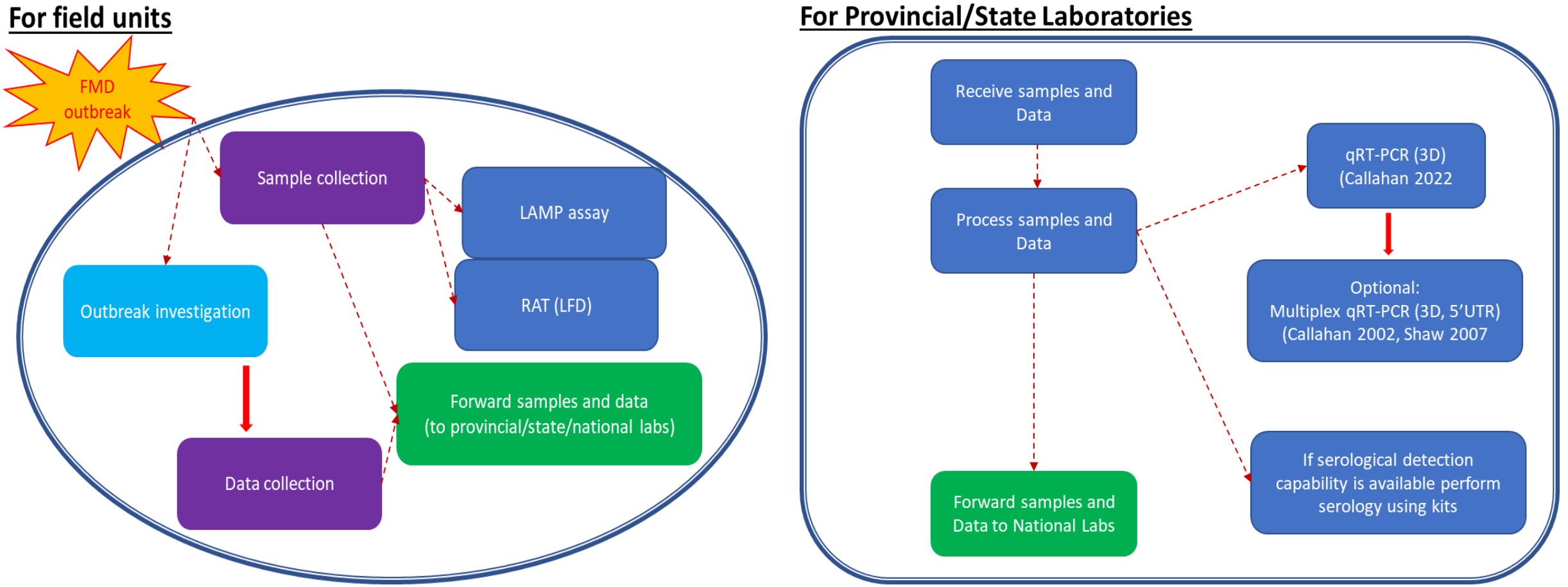
中华人民共和国农业农村部
Ministry of Agriculture and Rural Affairs of the People's Republic of China

NEW ZEALAND
FOREIGN AFFAIRS & TRADE
Aid Programme

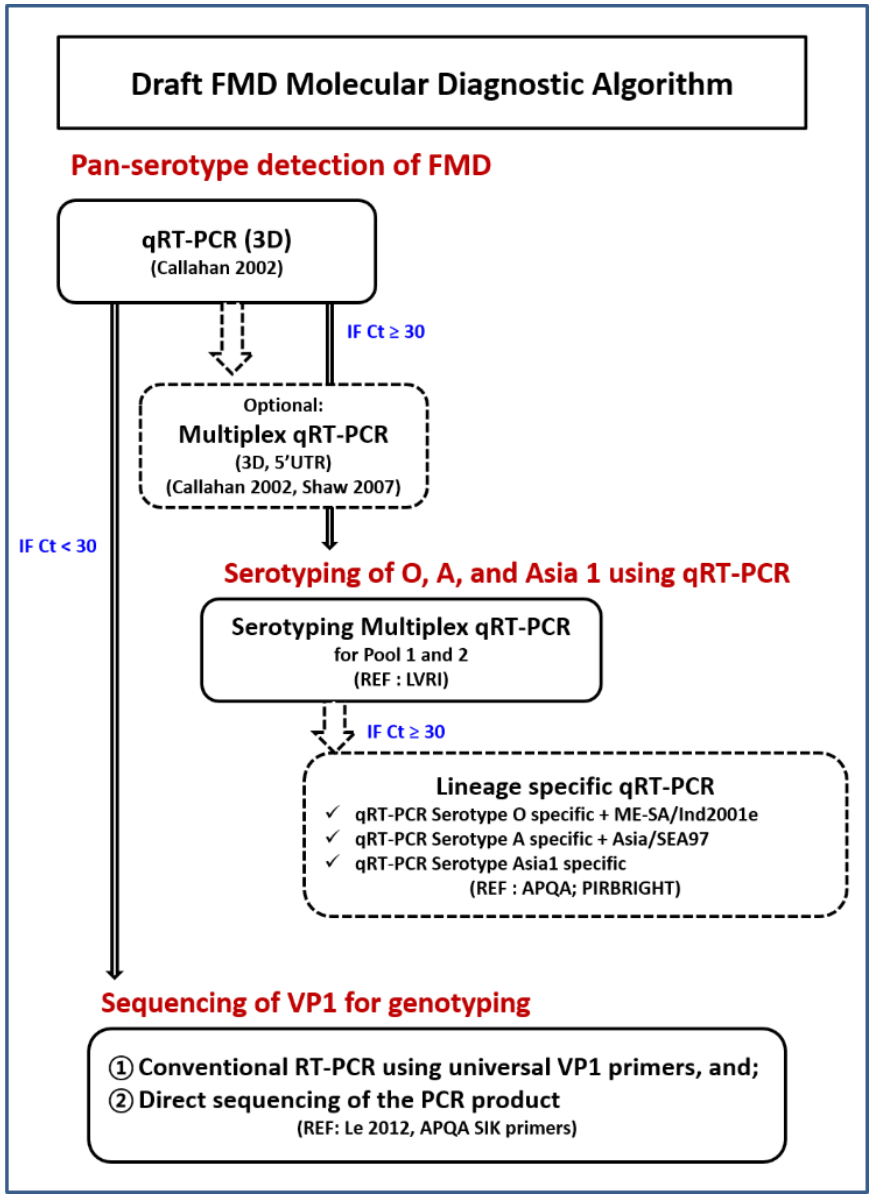
SEACFMD

CSIRO

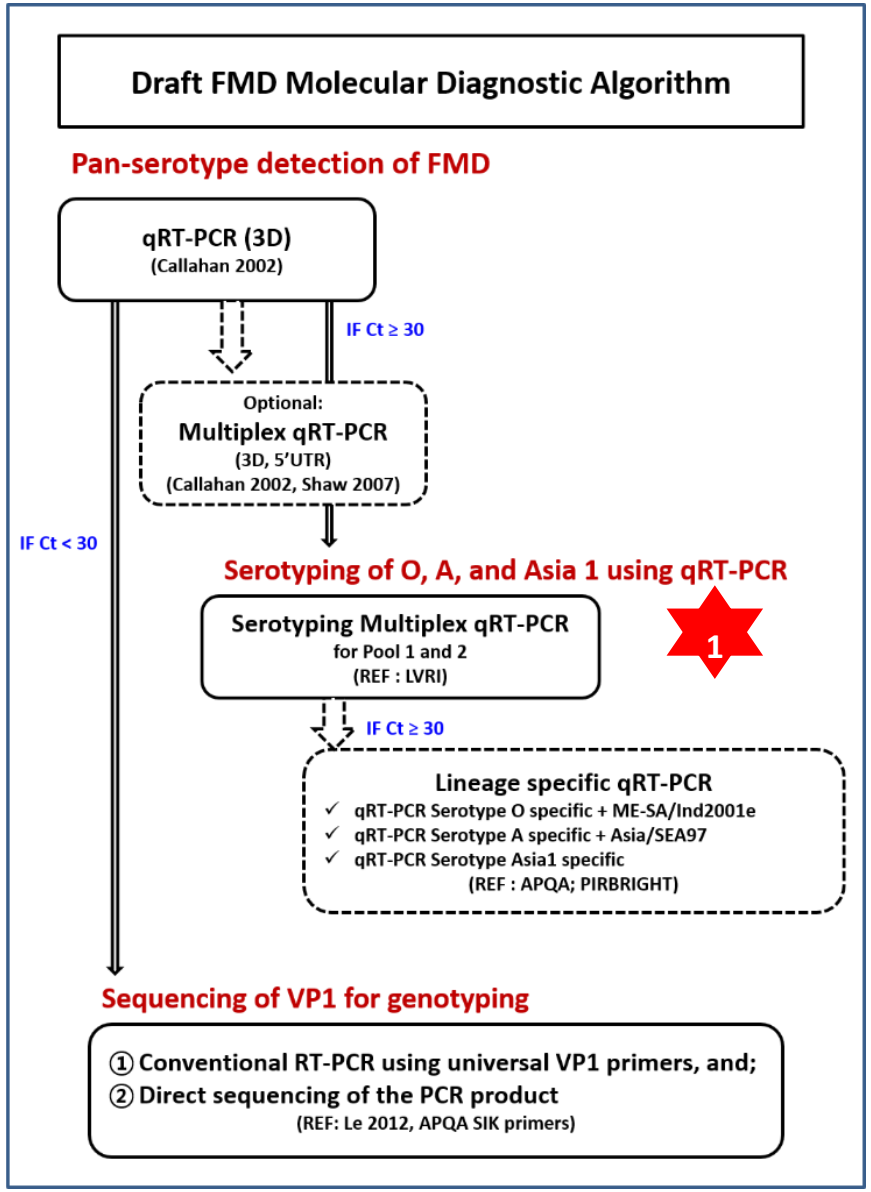
Diagnostic Algorithm for FMD laboratories in Southeast Asia



National / Reference Laboratories



Verification exercise for the proposed molecular diagnostic algorithm

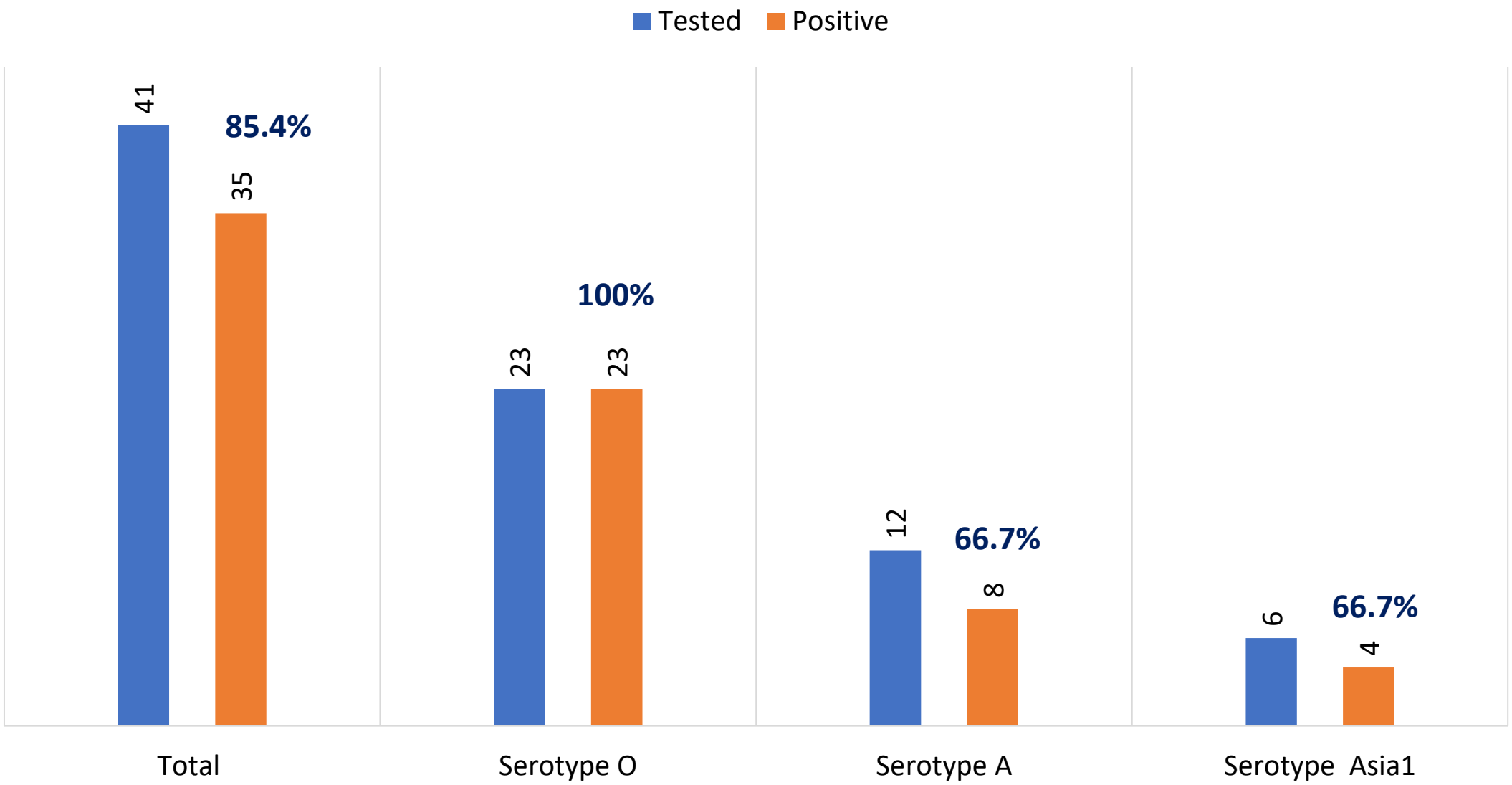


1. LVRI Multiple RT-qPCR
2. APQA Lineage specific RT-qPCR
3. Pirbright Lineage specific RT-qPCR
4. Le et al 2012 sequencing
5. APQA SIK sequencing

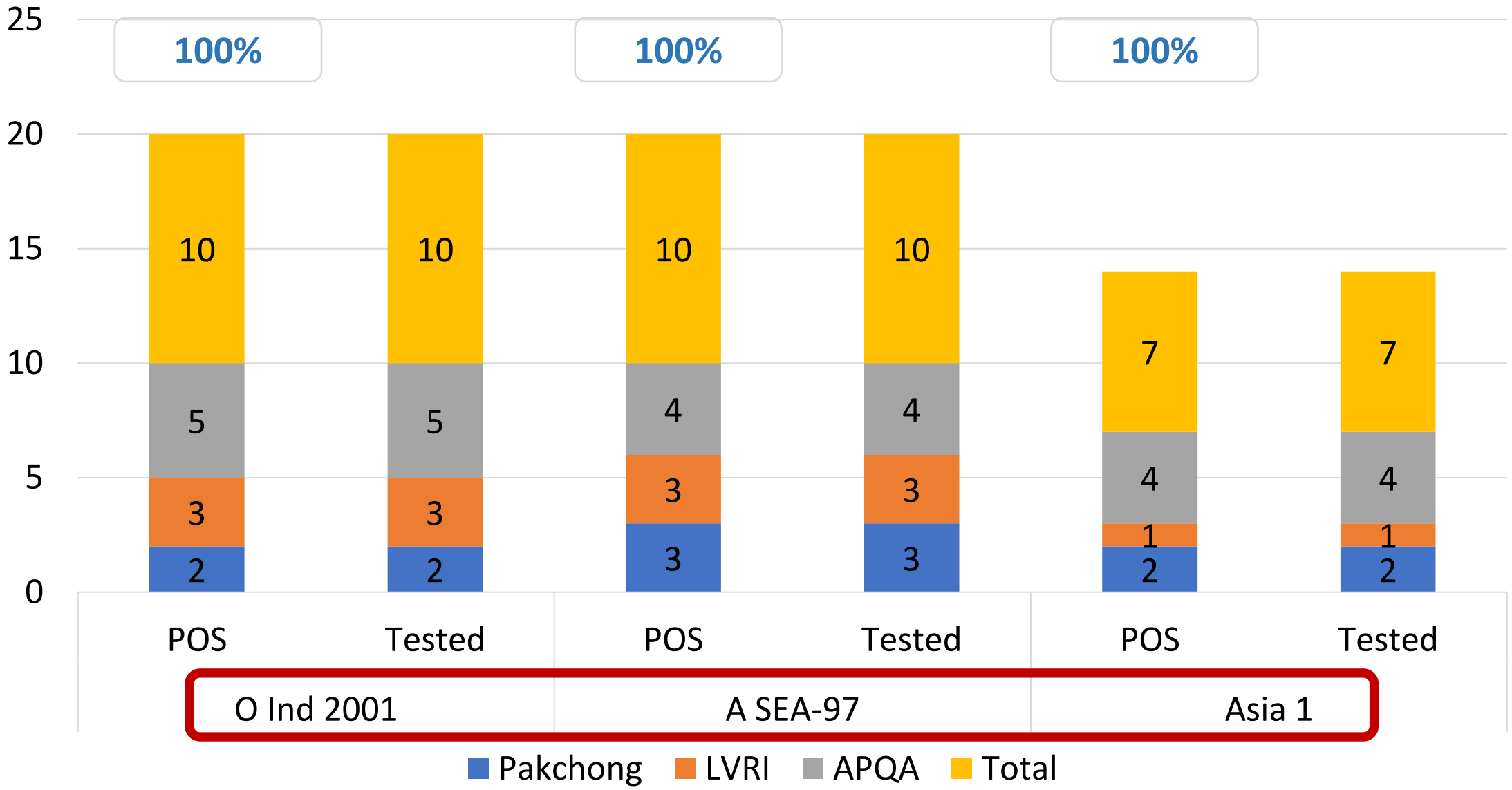
Progress in three OIE reference laboratories for FMD in Southeast Asia

	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**

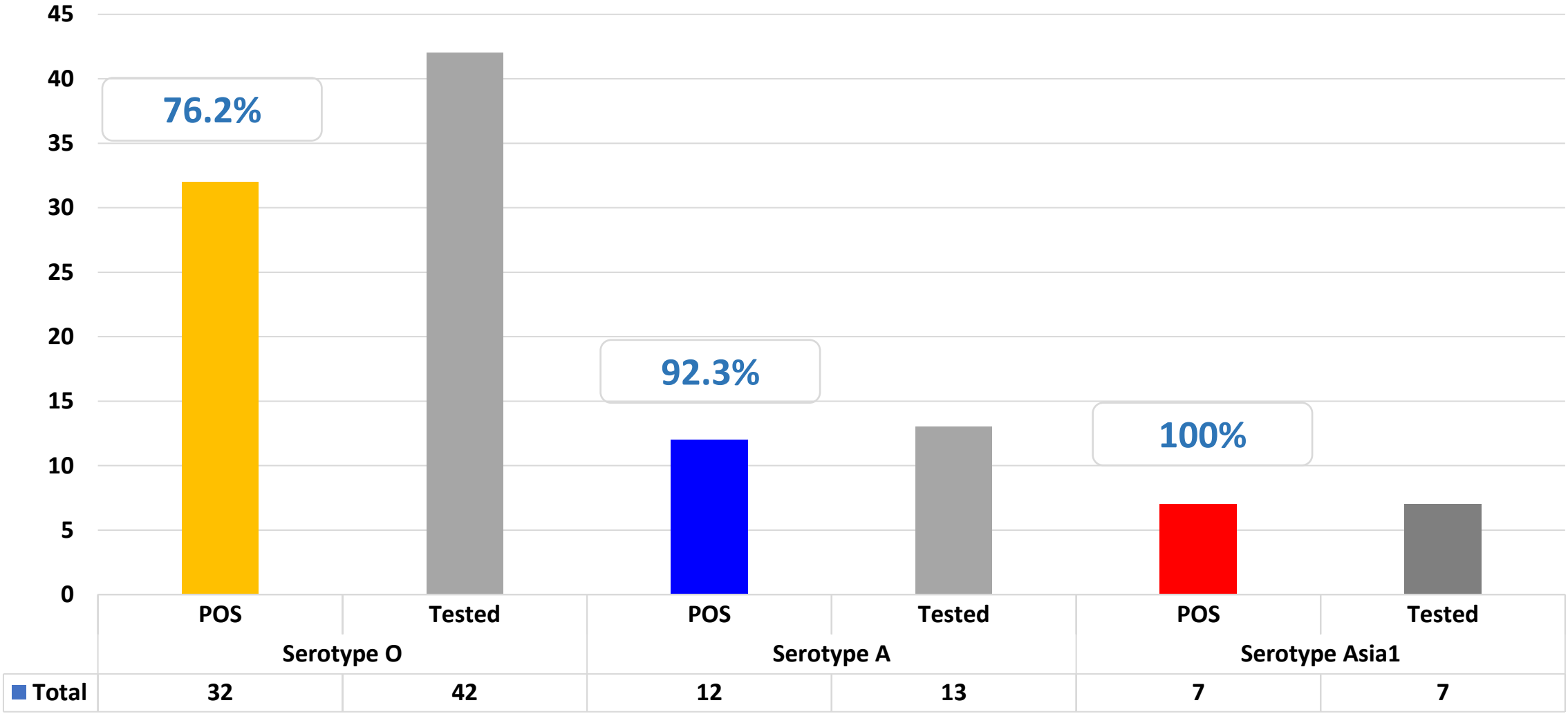
Serotyping (O, A, Asia1) Multiplex rRT-PCR



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

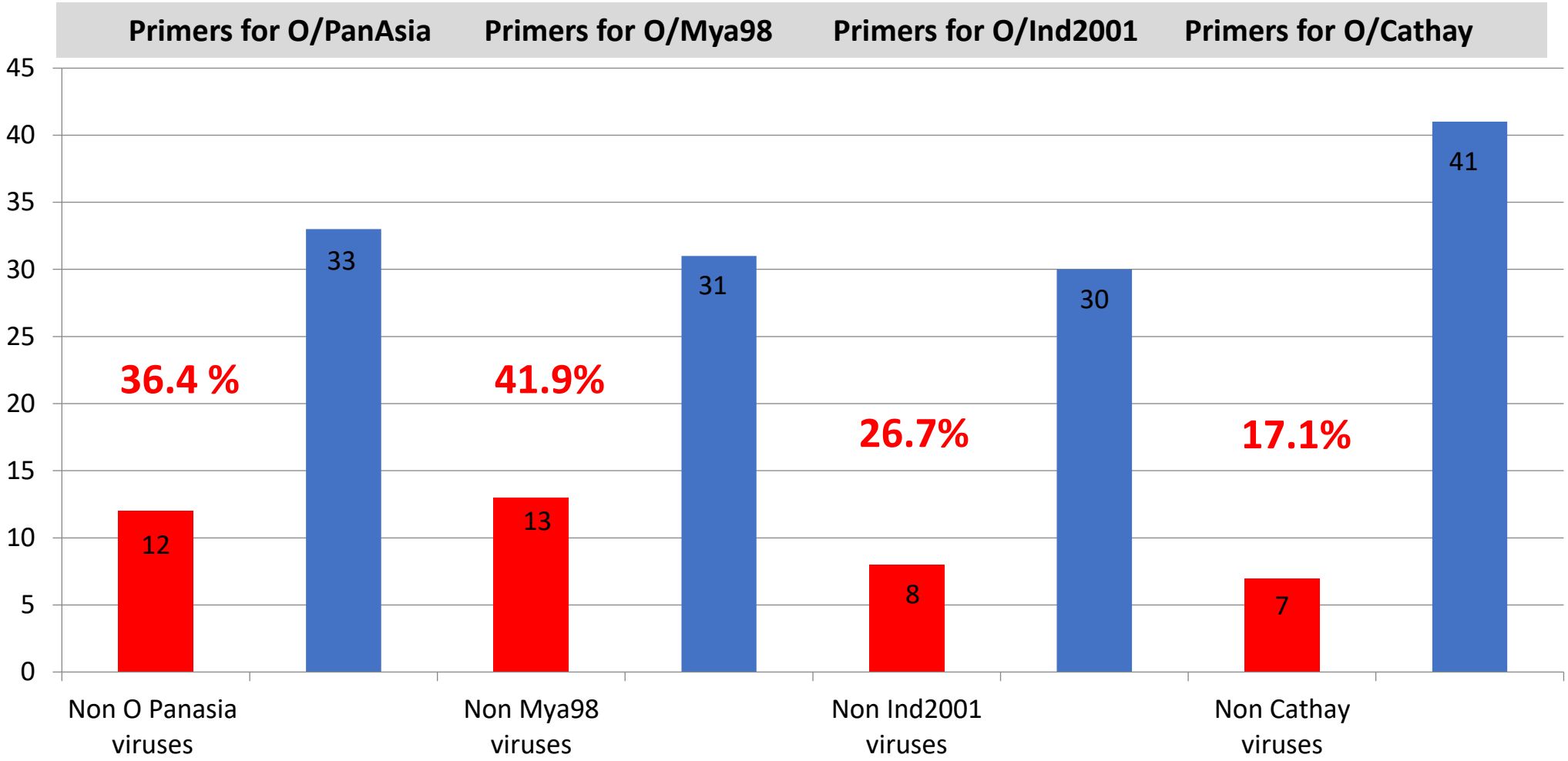


Lineage-specific Multiplex qRT-PCR (Pirbright)



Lineage specific qRT-PCR (Pirbright Institute)

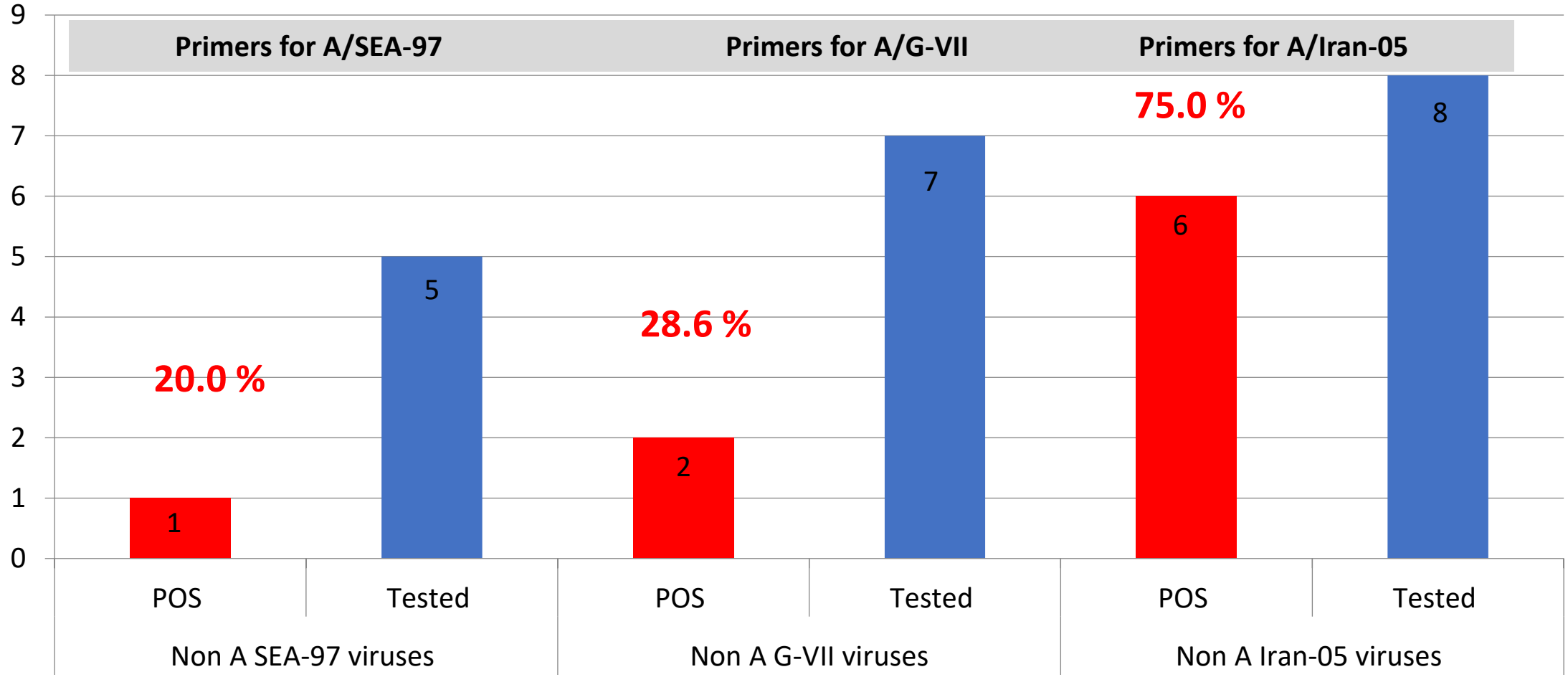
Cross reaction in lineages of Serotype O



Also detects Serotype A viruses

Lineage specific qRT-PCR (Pirbright Institute)

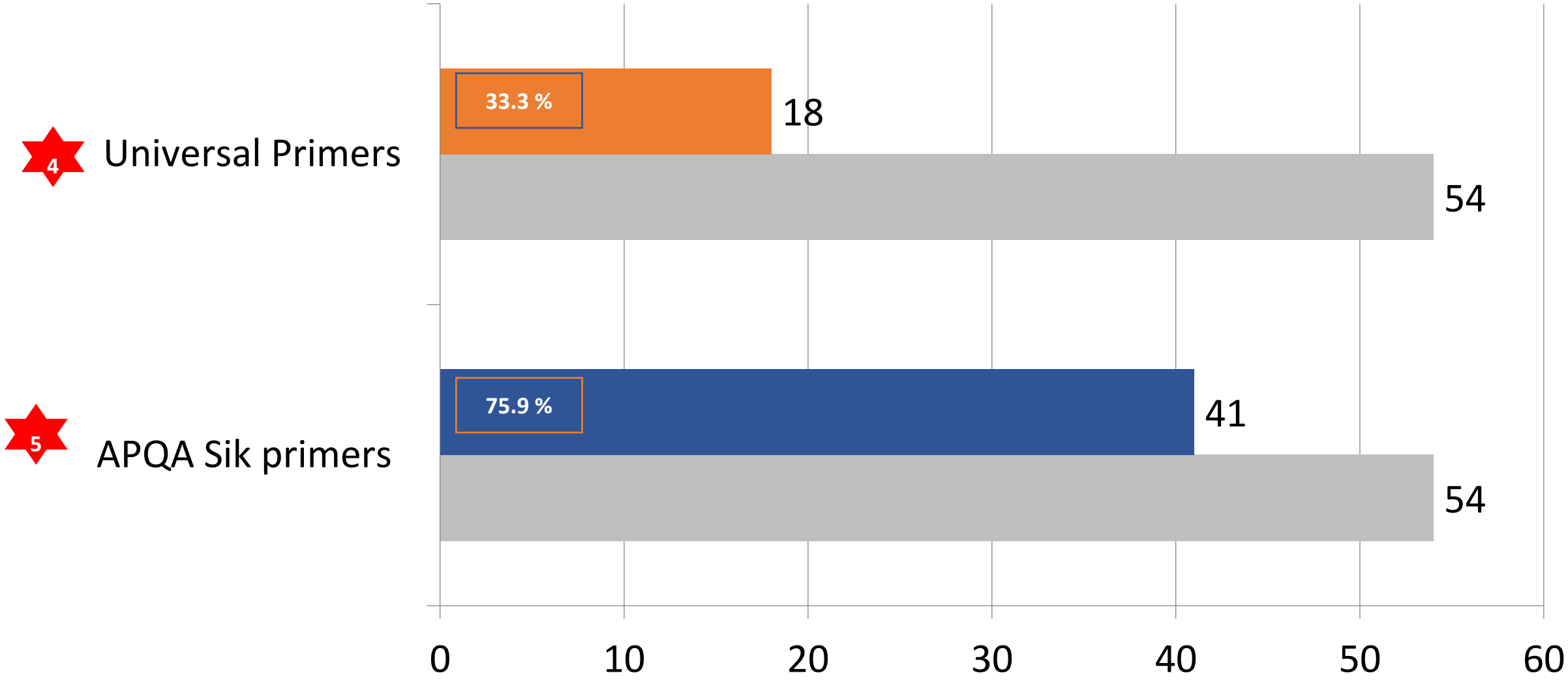
Cross reaction in lineages of Serotype A



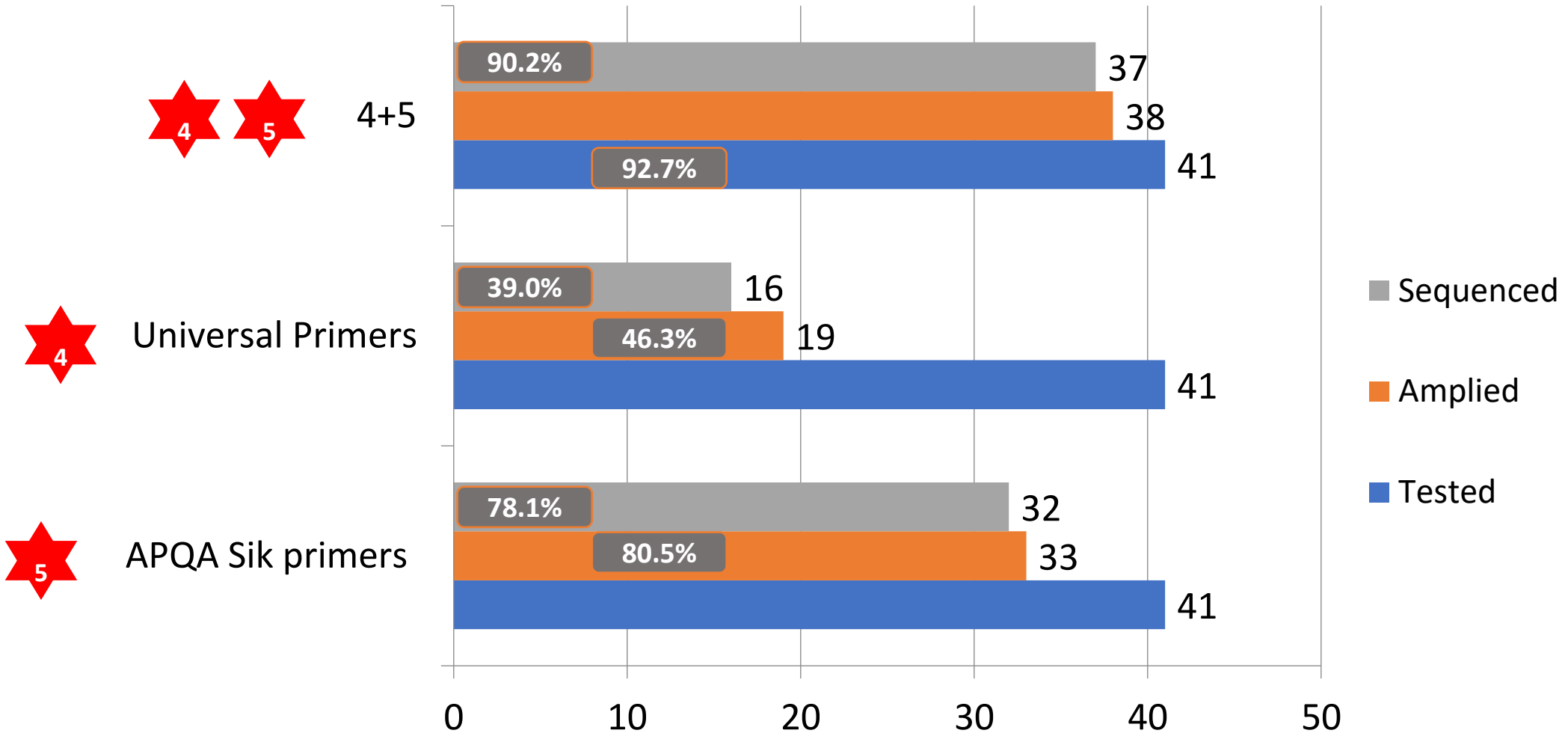
also detects Serotype O and Asia1

also detects Serotype O

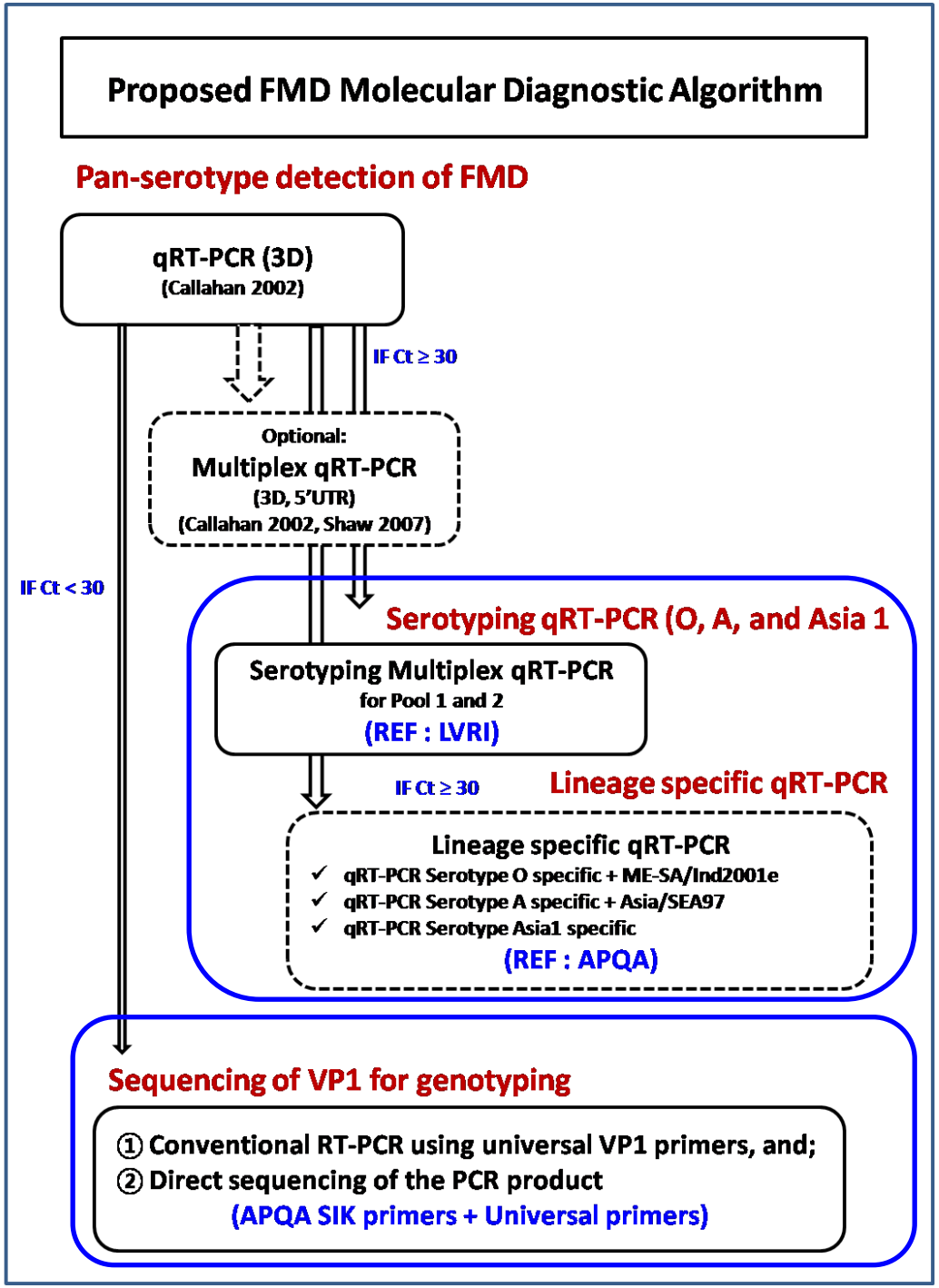
Sequencing VP1 for genotyping – results



Sequencing VP1 for genotyping – results



* Data from RRL-Pakchong and APQA


Proposed FMD Molecular Diagnostic Algorithm
Pan-serotype detection of FMD
qRT-PCR (3D)
(Callahan 2002)
IF Ct ≥ 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 ≥ 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

1

Piloting revised algorithm with more viruses

(> 30 viruses in each laboratory)

2

Inviting country FMD laboratories for piloting

(Myanmar, Viet Nam)

Group 1: Assay verification

Verification for the performance of existing assays with a new batch of reagents

- Optimise the performance (new vs. old reagents)
- Checker-board titrations of the antibodies and antigen
  - minimise cross-reactions (Ideally, the cross-reaction must be <10 percent).
  - Titration of antigen to establish linearity of antigen dilution.
- Strengthen assay performance by multiple testing of antigen
  - Batch testing at different time points
  - Inter-personnel comparison or day-to-day comparison
- Monitor the performance of IQC standards
  - Compare new set with existing reagents.
- Establish equivalence using 1-5 reference sera (high, moderate and low titres along with negative samples)
- Titrate every batch of commercial conjugate before use

Verification for new batches of commercial diagnostic kits or new kits introduced in the market

- Monitor the performance of IQC standards under the new set of reagents and compare with the existing reagents.
- Establish equivalence using 1-5 reference sera (high, moderate and low titres along with negative samples)

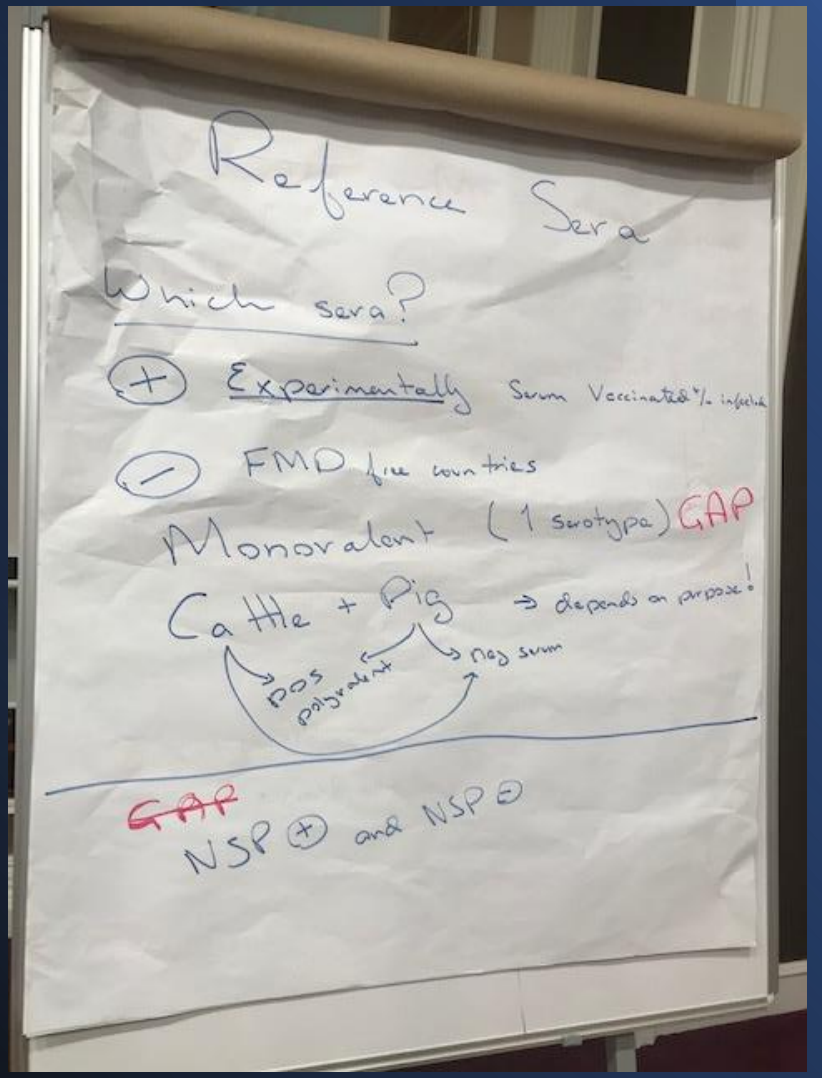
Additional comments

- Follow the ‘Westgard Rules’ while monitoring the IQC results.
- When IQCs are exhausted, establish the equivalence of the fresh batch of IQC with at least 5-10 runs before IQCs are changed to the fresh batch.
- Test the specificity and cross-reactions of Skim Milk Powders used in the blocking steps, if used.

Group 2: Monovalent Reference Serum for serological assays (VNT, LPBE, SPCE and NSP ELISA)

- **Optimal monovalent serum for reference panel:**
- Positive Serum:
  - Experimental serum from vaccinated, vaccinated/infected and/or infected sera
  - Monovalent serum (1 serotype/strain)*.
- *this is a gap; vaccine companies in the region supply bivalent/trivalent serum only.
- Negative Serum
  - FMD-free country without vaccination.
- Cattle and pig serum*
- Panel should include NSP negative and NSP positive serum.

- *this is dependent on the purpose of the testing and could be expanded.



Group 2: Monovalent Reference Serum for serological assays (VNT, LPBE, SPCE and NSP ELISA)

Monovalent serum available to the region for reference serum panel (WRL for FMD)

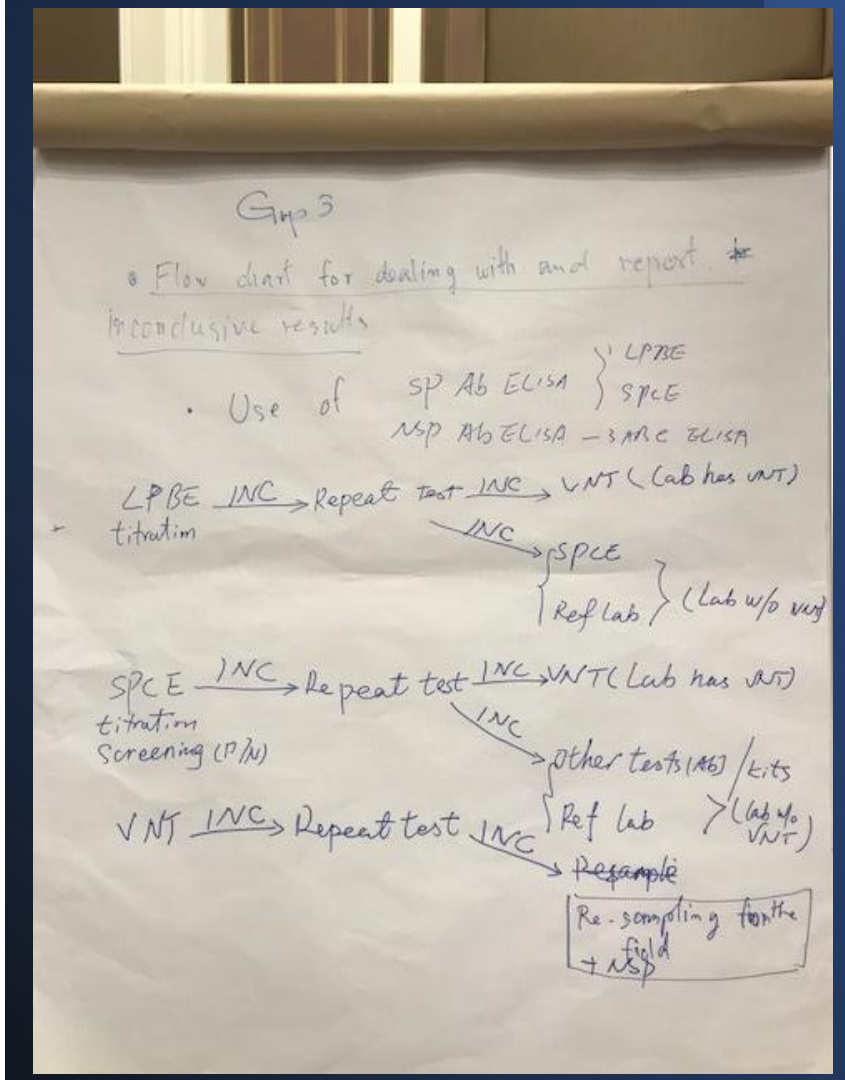
- Only cattle available now
- Except for the negative serum, two individual animal sera will be provided for each type of sera listed above. Fifty ml will be provided for each serum.
- For each type of sera, the following will be provided:
  - LPBE, SPCE, VNT and PrioCHECK results from WRL
  - If available, one NSP positive and one NSP negative sera
  - If available, high and mid-range sera determined by VNT
- The recommended control sera are in the tables.

Serotype O		Serotype Asia 1
O1 Manisa	A22 IRQ	Asia 1 Shamir
O 3039	A/MAY/97	
O/SKR*	A24**	Negative Cattle Serum

Thailand	Japan	China
O-3039 (or O MYA-98)	O1 Manisa	Needs to be confirmed
A/MAY/97	O-3039	
Asia 1 Shamir	A22 IRQ	

Group 3: Management and reporting of inconclusive results

- **<u>Inconclusive results are obtained in the following test methods:</u>**
- Serological assays for detecting antibodies against FMDV structural proteins (SP).
  - Liquid Phase Blocking ELISA (LPBE)
  - Solid Phase Competition ELISA (SPCE) and
  - Virus Neutralisation Test (VNT)
- Serological assays for detecting antibodies against non-structural proteins of FMDV (NSP): NSP- Ab ELISA
- LPBE (Titration): Repeat the assay / Perform VNT if available / Send sample to reference laboratory or test by SPCE
- SPCE (Titration, Screening; P/N): Repeat the assay / Perform VNT if available / Test using another set of antibodies (serotype-specific) or kit / Send to the reference laboratory for confirmation by VNT.
- VNT (for identification of exposure): Repeat the assay / Request for resampling from the field / Perform NSP-Ab ELSIA.
- NSP-Ab ELISA: Repeat the test/test with another kit or assay of a similar type. Probang sample can be tested by RT-qPCR or resampling after a week. The sample can also be sent to a reference laboratory for VNT and NSP-Ab ELISA confirmation.



Group 3: Management and reporting of inconclusive results

- **Assuring quality of VNT in Reference or National Laboratories**
- Testing the susceptibility of cells used in VNT (performed at least once in 3 months).
  - Use a well-characterized reference virus pool and check CPE at 24 and 48 hrs post-infection.
  - Establish susceptibility at different passage levels and set a maximum passage level for each cell type used.
  - Set up a 3-tier cell culture system with master stocks (MB) and working stocks (WB1 and WB2).
- Establish the titre of the reference control sera.
  - Include the controls in every run.
  - Monitory titre and establish moving averages.
- Virus monitoring
  - Monitor virus control titre in every assay.
  - Back titration of virus dilutions to confirm the virus dose (32-320 $TCID_{50}$/ml or 1.5-2.5 Log_{10} $TCID_{50}$/ml)
- Contamination in cell
  - Check for Mycoplasma contamination (every 6 months)
  - Observe for any physical change in cell growth, discolouration of media, contamination etc., on uninfected controls.