World Organisation for Animal Health
Founded as OIE

SEACFMD

LVRI

Sponsored by People's Republic of China

SEACFMD Laboratory Network Meeting

Regional Expert Group for FMD diagnosis

Bolortuya, P
WOAH SRR SEA

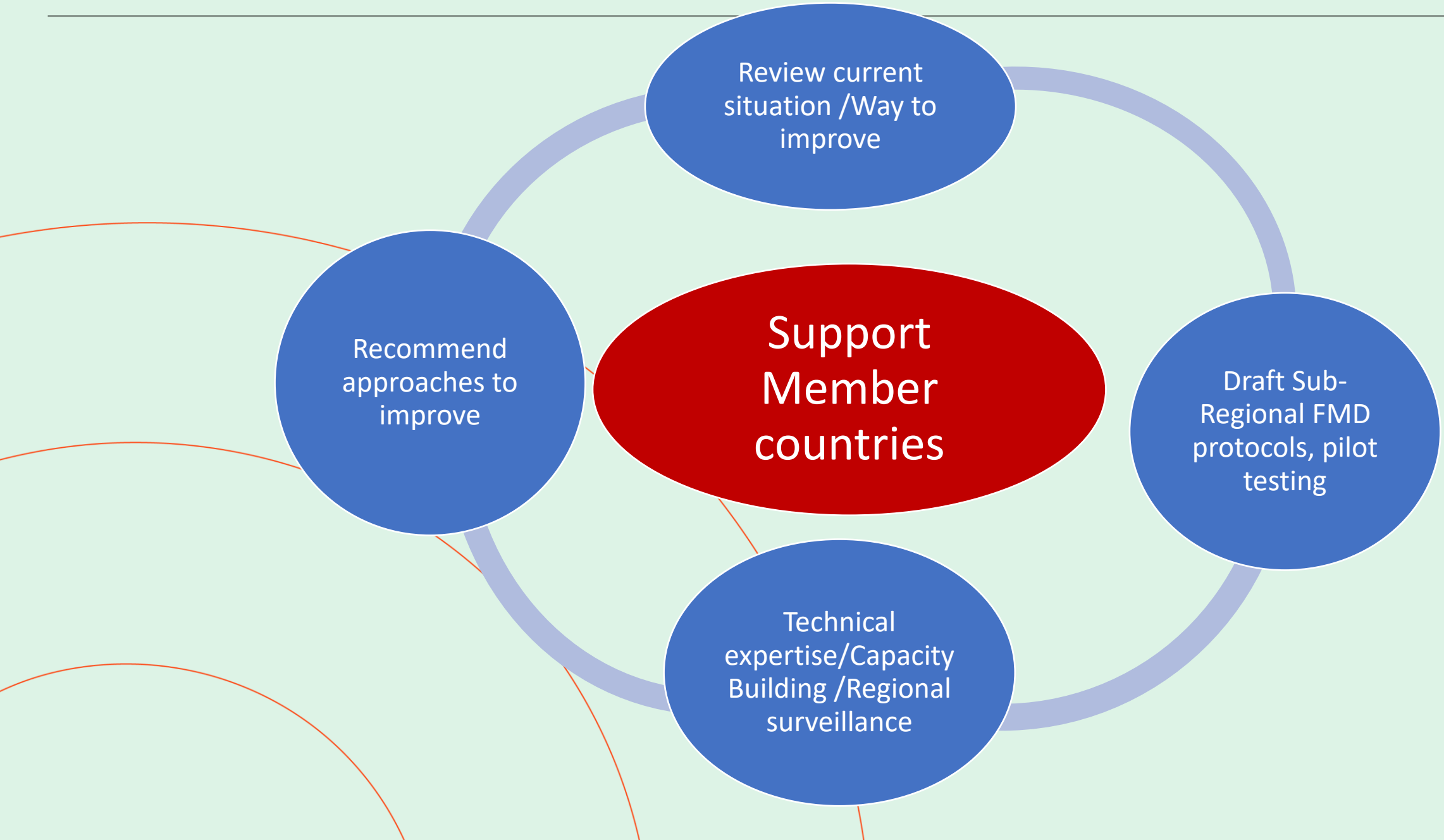
24-25 October 2023
Lanzhou, China

Establishment of FMD Laboratory
Regional Expert Group

In collaboration with FAORAP

10th Lab-TAG meeting was held on 29-31 October 2018, Singapore
6th ASEAN Laboratory Directors' Forum meeting held in 2018
OIE SEACFMD LabNet meeting in 2017

- to evaluate the current situation on FMD (molecular) diagnosis and other relevant issues, including vaccine matching and post vaccination monitoring
- using more advanced tests such as PCR and SPCE for routine diagnosis to complement the traditional assays including antigen ELISA and serological LP ELISA.


Review current situation /Way to improve
Draft Sub-Regional FMD protocols, pilot testing
Technical expertise/Capacity Building /Regional surveillance
Recommend approaches to improve
Support Member countries

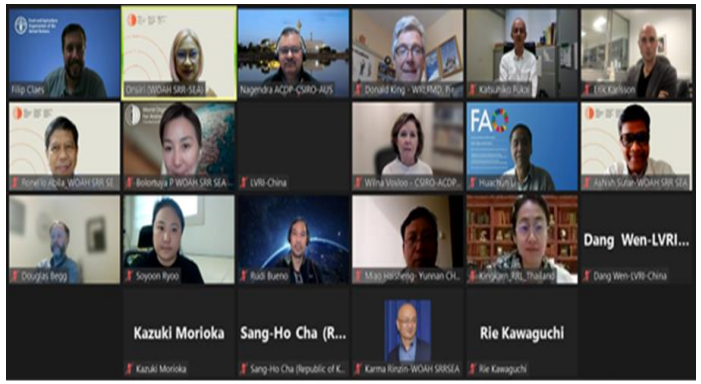
Focus on Molecular diagnosis

Serological assays

Review and follow up on the preceding meeting

Participating laboratories:

- WRLFMD
- Lanzhou Veterinary Research Institute (LVRI), China
- FMD Diagnostic Laboratory, Pakchong FMD, Thailand
- Australian Centre for Disease Preparedness (ACDP), Australia
- Animal and Plant Quarantine Agency (APQA), Korea
- National Institute of Animal Health (NIAH), Japan
- Yunnan Animal Science and Veterinary Institute (Yunnan ASVI), China



3rd REG meeting on FMD, Virtual Meeting June 2022

1st **Regional Expert Group Meeting on Foot and Mouth Disease in May 2019**

Development of FMD field sampling SOPs to improve lab diagnosis yields

Sample collection

Sample preservation

Sample submission (transportation)

- **A summary table for FMD sampling for lab diagnosis (all species)** ← **LVRI (all REG members)**
- **A draft regional protocol for probang samples (ruminants)** ← **LVRI (YASVI+Pakchong)**
- **A draft regional protocol for swab samples (all species)** ← **AAHL (APQA+LVRI)**
- **A draft regional protocol for FTA Cards (all types of samples)** ← **AAHL**

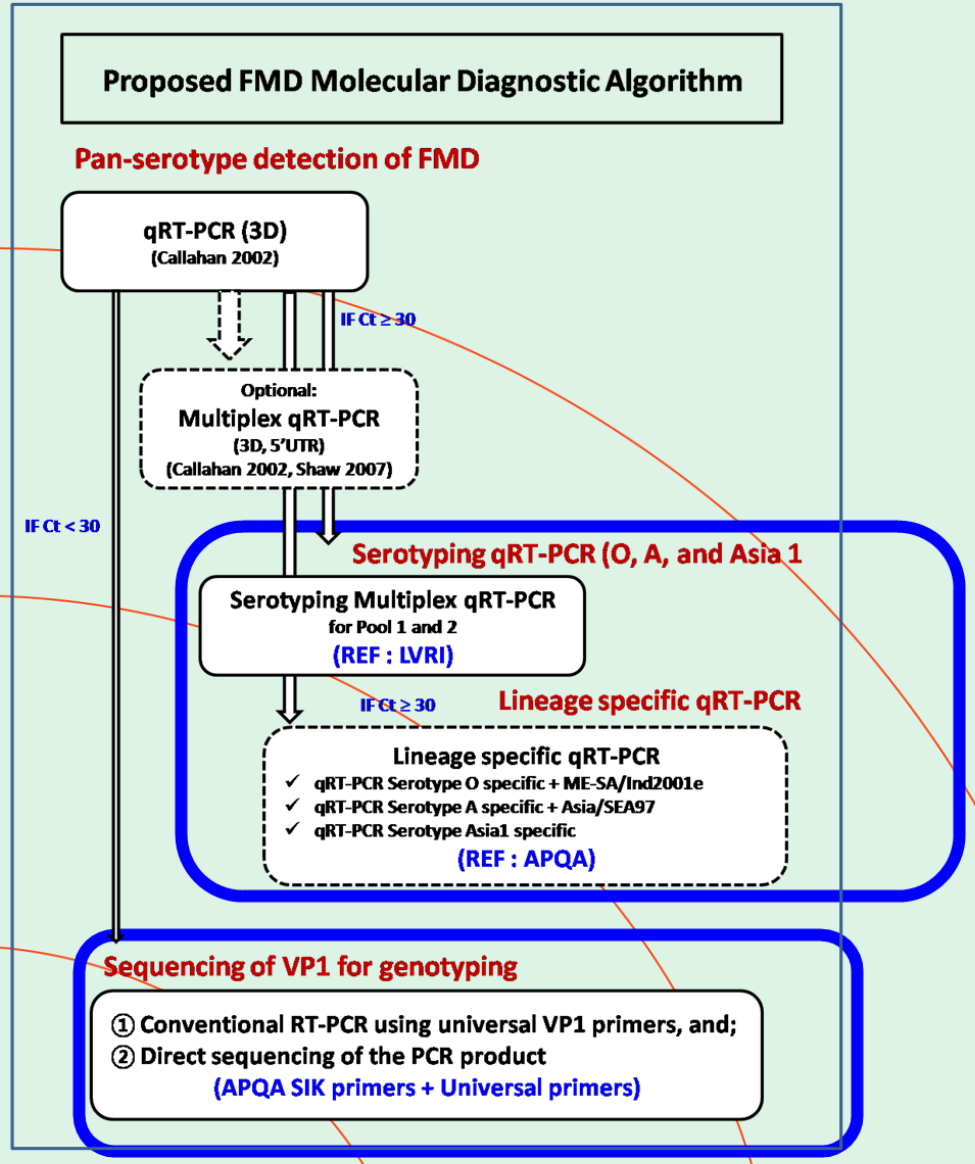
Virus isolation

Agree not to explore a regional technical guideline on cell culture and VI

Molecular typing

Discussed at Session 1

FMD Molecular Diagnostic Scheme to allow rapid virus identification and characterization



Pilot testing:

Malaysia
Vietnam

Planned:

Vietnam
Myanmar

Result from Pilot testing

Results of VP1 sequencing **(Le 2012 protocol)**

Result sheet for validation of universal amplification of VP1 of FMDV isolates in SEA

Lab	No. of samples	No. of samples amplified	No. of samples sequenced	Serotype		Genotype								Other information
				O	A	Asia 1	O Panasia	O Mya98	O Ind 2001	O Cathay	A SEA-97	A G-VII	Asia 1 lineage	
APQA	29* (O:16,A:9,Asia1:4)	9	9	4/16	1/9	4/4	2/5	0/3	1/5	0/1	0/4	1/2	4/4	O/EA-2 (1/1)
Pakchong FMD Lab	12 (O=7,A=3, Asia1=2)	Positive band (9)	8	3	3	2	2	1	-	-	3	-	2	
LVRI	13	2	2	10	2	1	0(2)	0(4)	1(2)					

Results of VP1 sequencing **(APQA protocol)**

Result sheet for validation of SIK amplification of VP1 of FMDV isolates in SEA

Lab	No. of samples	No. of samples amplified	No. of samples sequenced	Serotype		Genotype			
				O	A	Asia 1	O Panasia	O Mya98	O Ind 2001
APQA	29* (O:16,A:9,Asia1:4)	25	25	15/16	8/9	2/4	5/5	2/3	5/5
Pakchong FMD Lab	12 (O=7,A=3, Asia1=2)	Positive band (7)	7	3	2	2	1	1	1
LVRI	13	9	9	10	2	1	1(2)	3(4)	2(2)

Results of specific real-time RT-PCR **(Pirbright protocol)**

Result sheet for validation of lineage specific real-time RT-PCR using FMDV isolates in SEA

Lab	No. of samples	No. of samples positive	No. of samples sequenced	Serotype			Genotype							Other information
				O	A	Asia 1	O Panasia	O Mya98	O Ind 2001	O Cathay	A SEA-97	A G-VII	Asia 1 lineage	
APQA	29* (O:16,A:9,Asia1:4)						4/5 Cross rx:8†	3/3 Cross rx:6†	4/5 Cross rx:6†	1/1	3/3 Cross rx:8†	2/2 Cross rx:2†	4/4	†See attached file
Pakchong FMD Lab	12 (O=7,A=3, Asia1=2)	7 (O=2,A=3,Asia1=2)		2	3	2	ND	ND	2 (No cross reaction)	ND	3 (No cross reaction)	ND	2 (No cross reaction	Sets of primer-probe from APQA for O/Ind2001, A/Sea-97 and Asia1 are good lineage specific with each sample.
LVRI	25			21	3	1	1(3) 2(Mya-98)	4(8)	5(7)	3(3)	2(3)	0	1(1)	

Results of Duplex (Serotype/lineage specific) real-time RT-PCR (APQA protocol)

Lab	No. of samples	No. of samples positive		Serotype			Genotype							Other information
				O	A	Asia 1	O Panasia	O Mya98	O Ind 2001	O Cathay	A SEA-97	A G-VII	Asia 1 lineage	
APQA	29* (O:16,A:9,Asia1:4)	28		15/16	9/9	4/4	0/5	0/3	5/5	0/1	3/3	0/2	4/4	O/EA-2 (0/1) O/WA (0/1) A/Others (0/3)

Group 1: Assay verification

Verification for performance of existing assays with 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.

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

- Only cattle available now

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

- Except for the negative serum, two individual animal sera will be provided for each of the sera types 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
- Following control sera are recommended

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

Inconclusive results are obtained in the following test methods:

- Serological assays for detecting antibodies against structural proteins of FMDV (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 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 similar type. Probang sample can be tested by RT-qPCR or resampling can be done after a week. The sample can also be sent to a reference laboratory for confirmation with VNT and NSP-Ab ELISA.

- **Assuring quality of VNT in Reference or National Laboratories**
- Testing the susceptibility of cells used in VNT (perform at least once in 3 months).
  - Using a well characterized reference virus pools and check CPE at 24 & 48 hrs post infection.
  - Establish susceptibility at different passage levels and set a maximum passage levels for each cell types 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, discoloration of media, contamination etc on uninfected controls.

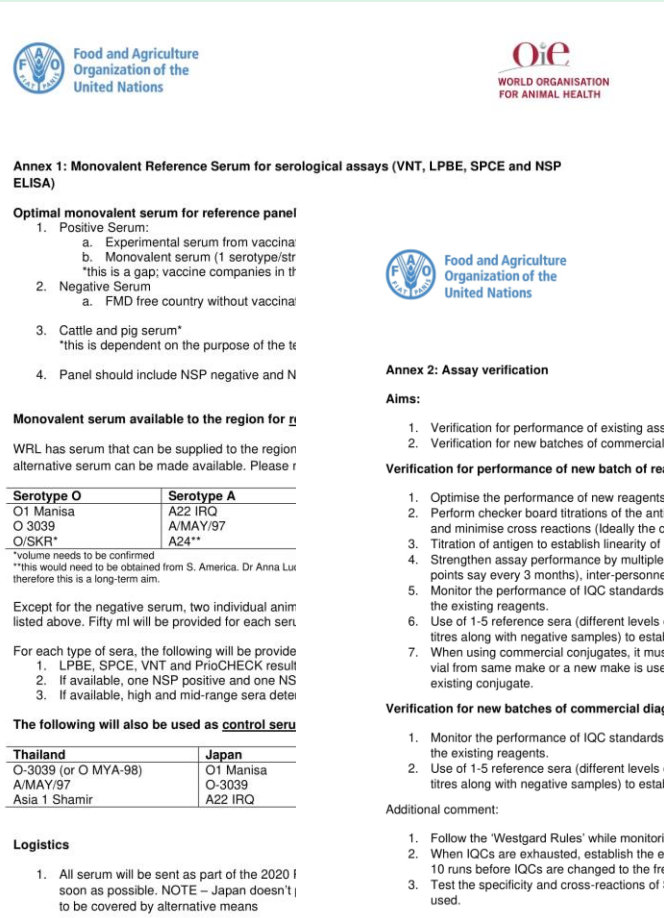
Recommendations:

To measure/examine immune responses against the SP or NSP of FMDV for different diagnostic purposes:

- To study antibody levels in individual animals or herds for post-vaccination monitoring(PVM), the REG recommends using SP ELISA (LPBE or SPCE) or VNT. Antigen should be selected with support from the vaccine and ELISA reagents producers.
- To study prevalence of FMDV infection (serosurveillance), the REG recommends using NSP ELISA together with SP ELISA and/or VNT.
- To identify FMDV infected animals and the serotype of infected virus, the REG recommends testing the animal by NSP ELISA together with SP ELISA (LPBE or SPCE). Given the cross-reactivity between different FMDV serotypes is common in SP ELISA (LPBE or SPCE), the REG recommends interpreting the serotype prevalence data from such tests with caution. If positive in SP ELISA, confirmatory testing by VNT is required.
- For vaccine-matching study, the REG recommends conducting VNT or LPBE at capable OIE reference labs only.

To improve verification of reagents quality and assay performance:

- To verify performance of a new batch of reagents to replace existing reagents for a validated diagnostic assay, the REG recommends optimising the performance of new reagents with old reagents and control samples;
- To verify performance of new assays, the REG recommends using IQC samples and reference sera samples;
- To monitor performance of serological assays (VNT, SP and NSP ELISAs), the REG recommends using monovalent reference serum panel;
- For inconclusive results from serological assays, the REG recommends developing a systematic approach to further verify the results.



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Annex 1: Monovalent Reference Serum for serological assays (VNT, LPBE, SPCE and NSP ELISA)

Optimal monovalent serum for reference panel

1. Positive Serum:
   a. Experimental serum from vaccina
   b. Monovalent serum (1 serotype/str
   *this is a gap; vaccine companies in th
2. Negative Serum
   a. FMD free country without vaccina
3. Cattle and pig serum*
   *this is dependent on the purpose of the te
4. Panel should include NSP negative and N

Monovalent serum available to the region for r

WRL has serum that can be supplied to the region
alternative serum can be made available. Please r

Serotype O	Serotype A
O1 Manisa	A22 IRQ
O 3039	A/MAY/97
O/SKR*	A24**

*volume needs to be confirmed
**this would need to be obtained from S. America. Dr Anna Luc
therefore this is a long-term aim.

Except for the negative serum, two individual anim
listed above. Fifty ml will be provided for each seru

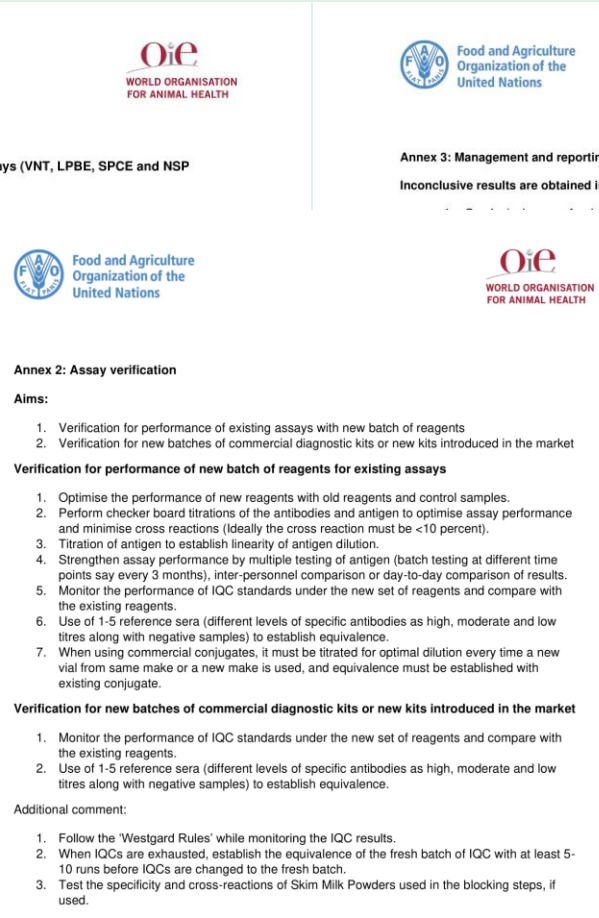
For each type of sera, the following will be provide
1. LPBE, SPCE, VNT and PrioCHECK result
2. If available, one NSP positive and one NS
3. If available, high and mid-range sera dete

The following will also be used as control seru

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

Logistics

1. All serum will be sent as part of the 2020 f
   soon as possible. NOTE – Japan doesn't p
   to be covered by alternative means



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Annex 2: Assay verification

Aims:

1. Verification for performance of existing assays with new batch of reagents
2. Verification for new batches of commercial diagnostic kits or new kits introduced in the market

Verification for performance of new batch of reagents for existing assays

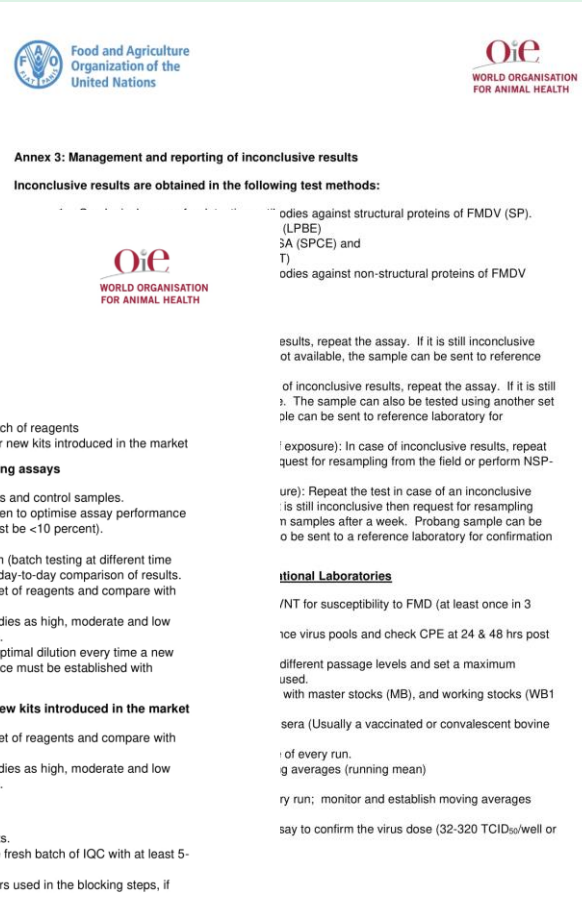
1. Optimise the performance of new reagents with old reagents and control samples.
2. Perform checker board titrations of the antibodies and antigen to optimise assay performance and minimise cross reactions (Ideally the cross reaction must be <10 percent).
3. Titration of antigen to establish linearity of antigen dilution.
4. Strengthen assay performance by multiple testing of antigen (batch testing at different time points say every 3 months), inter-personnel comparison or day-to-day comparison of results.
5. Monitor the performance of IQC standards under the new set of reagents and compare with the existing reagents.
6. Use of 1-5 reference sera (different levels of specific antibodies as high, moderate and low titres along with negative samples) to establish equivalence.
7. When using commercial conjugates, it must be titrated for optimal dilution every time a new vial from same make or a new make is used, and equivalence must be established with existing conjugate.

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

1. Monitor the performance of IQC standards under the new set of reagents and compare with the existing reagents.
2. Use of 1-5 reference sera (different levels of specific antibodies as high, moderate and low titres along with negative samples) to establish equivalence.

Additional comment:

1. Follow the 'Westgard Rules' while monitoring the IQC results.
2. 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.
3. Test the specificity and cross-reactions of Skim Milk Powders used in the blocking steps, if used.



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Annex 3: Management and reporting of inconclusive results

Inconclusive results are obtained in the following test methods:

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(LPBE)
3A (SPCE) and
T)
odies against non-structural proteins of FMDV

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exposure): In case of inconclusive results, repeat
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is still inconclusive then request for resampling
n samples after a week. Probang sample can be
o be sent to a reference laboratory for confirmation

itional Laboratories

/NT for susceptibility to FMD (at least once in 3

ice virus pools and check CPE at 24 & 48 hrs post

different passage levels and set a maximum
used.
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sera (Usually a vaccinated or convalescent bovine

of every run.
g averages (running mean)

ry run; monitor and establish moving averages

say to confirm the virus dose (32-320 $TCID_{50}$/well or

10

9

THANK YOU FOR YOUR ATTENTION