2nd Regional Expert Group for FMD in SEA Meeting

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Workshop 2: Recommendations to improve verification of reagents quality and serology assay performance

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SEACFMD

Serology methods for different diagnostic purposes

- To measure/examine immune responses against the SP or NSP of FMDV.
- Study antibody levels in individual animals or herds for post-vaccination monitoring(PVM), the REG recommends to use SP ELISA (LPBE or SPCE) or VNT. Choose antigen with support from the vaccine and ELISA reagents producers.
- To study prevalence of FMDV infection (serosurveillance), the REG recommends to use NSP ELISA together with SP ELISA and/or VNT.
- To identify FMDV historical infected animals and the serotype of infected virus, the REG recommends to test the animal by NSP ELISA together with SP ELISA (LPBE or SPCE). Given cross-reactivity between different FMDV serotypes is common in SP ELISA (LPBE or SPCE), the REG recommends to interpret 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 to conduct VNT or LPBE at capable OIE reference labs only.

Group 1: Assay verification

Verification for performance of existing assays with new batch of reagents

- Optimise the performance of 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 using 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.

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

- Negative Serum
  - FMD free country without vaccination.
- Cattle and pig serum
- Panel should include NSP negative and NSP positive serum.

Monovalent serum available to the region for <u>reference serum panel from WRL for FMD</u>

- 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. 50 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	O/BY/2010
A/MAY/97	O-3039	A/WH/09
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): 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 from the same animal can be collected and 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.

<u>Assuring quality of VNT in Reference or National Laboratories</u>

- Testing the susceptibility of cells used in VNT (e.g every 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 (running mean).
- Virus monitoring
  - Include virus control in every plate.
  - Back titration of virus dilutions to confirm the virus dose (32-320 $TCID_{50}$/ 50µl or 1.5-2.5 Log_{10} $TCID_{50}$/50µl)
- Be ware of contamination in cell culture
  - Check for Mycoplasma contamination (every 6 months)
  - Observe for any physical change in cell growth and uninfected controls: discoloration or contamination etc