FOOT AND MOUTH DISEASE:

DETECTION OF ANTIBODIES AGAINST NON-STRUCTURAL VIRAL PROTEINS

NRM number 013

ISSUED BY The Veterinary and Public Health Test Standardisation Group
a sub group of the UK Surveillance Group for Diseases and Infections
of Animals (SGDIA)

Whilst every care has been taken in the preparation of this publication, the group cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field of laboratory diagnostics and specialist advice should be obtained where necessary. Please refer to the text under “Limitations”.

The procedures described are those currently used as at 9 August 2007.

UK NATIONAL HARMONISED PROCEDURE FOR THE DETECTION OF NSP ANTIBODIES TO FMD VIRUS

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AMENDMENT PROCEDURE

Controlled document reference | NRM 013
Controlled document title | Foot and Mouth Disease : Detection of Antibodies against non-structural viral proteins

The current approved version is always the one on the SGDIA website (http://www.defra.gov.uk/animalh/diseases/vetsurveillance/sgdia/standardisation.htm).

Holders of working copies should ensure they are up to date in respect of amendments in this table.

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REVIEW HISTORY

This National Reference Method should be reviewed annually by appropriate experts to ensure that it is in line with current knowledge and practice. Any identified need for revision should be referred to the Veterinary Test Standardisation Group for action.

Publication Date: 9 August 2007

Next review due 9 August 2008
Review complete
Website updated
UK NATIONAL REFERENCE METHOD
FOR THE INVESTIGATION OF ANTIBODIES TO
NON-STRUCTURAL PROTEINS OF FOOT AND MOUTH DISEASE VIRUS

Type of specimens or samples: Serum samples

1. INTRODUCTION

1.1 Scope

This National Reference Method (NRM) describes the application of a commercial test kit to the detection of antibodies to non-structural proteins of foot and mouth disease virus (FMDV). It may be used in the UK only under the direction of the relevant animal disease control authorities and on advice from the National Reference Laboratory at the Institute for Animal Health (IAH), Pirbright (see Appendix I).

1.2 Background

FMDV non-structural proteins (NSP) are only produced when the virus replicates in permissive cells. Modern FMDV vaccines produced by reputable manufacturers are highly purified and inactivated. They cannot replicate, and have, to date, shown no evidence of containing co-purified 3ABC NSP. Therefore seroconversion of susceptible animals to 3ABC is considered indicative of FMDV infection. FMDV non-structural immunodeterminants, unlike structural ones, are non-serotype specific.

The Ceditest® FMDV-NS is a commercially available kit produced by Cedi Diagnostics B.V. It is a blocking ELISA which detects antibodies against foot and mouth disease virus (FMDV) non-structural proteins. ELISA plates supplied in the kit have been coated with 3ABC specific monoclonal antibodies followed by incubation with the 3ABC protein. This kit can be used not only to determine FMDV infection regardless of serotype but also discriminates between infected and vaccinated animals. It is stated that Ceditest FMDV-NS kits are used for detecting anti-NS antibodies in sheep, cattle, goat and pig serum though, theoretically, they should be applicable to all animal species.

It should be noted that antibody to 3ABC appears later in infection than antibody to the structural proteins and that the duration of the response has not been elucidated. Due to low sensitivity of this assay, especially early in infection, the assay is recommended only for screening herds of animals, not on an individual animal basis.

1.3 Limitations

Foot and mouth disease is a notifiable disease and all testing is regulated by the national competent authorities. Tests are normally carried out only at the National Reference Laboratory at the Institute for Animal Health, Pirbright (Appendix 1). Contingency plans for large scale testing in the event of an outbreak require the support of other designated laboratories. This National Reference Method has been prepared in consultation with experts at Pirbright and should only be used under the scientific direction of the Pirbright laboratory.

The procedures used for collection and transport of specimens or samples from healthy or clinically affected patients or animals, or post mortem, are critically important for successful laboratory analyses. These must be done in accordance with current best practice.
It is essential that laboratories have evidence of adequate validation of methods, equipment and commercial and in-house test procedures demonstrating that they are fit for purpose. Internal and external quality assurance procedures should be in place.

Where commercial reagents or equipment are suggested, the information is provided solely to assist scientists to identify suitable sources of such items. No specific endorsement or approval is intended and equivalents from other manufacturers may be equally suited.

Local laboratory managers have the primary responsibility for risk assessments and safety management when conducting the procedures described in this document. Expert advice should be sought wherever considered necessary.

The authors of this document, and the Veterinary and Public Health Test Standardisation Group that has authorised it, have no control over the implementation of sampling or testing procedures. The document is intended solely as a general resource for practising professionals in the field of laboratory diagnostics and specialist advice should be obtained where necessary. It is expected that individual laboratories will develop their own Standard Operating Procedures (SOPs) adapting this NRM to the local situation and Quality System requirements. No changes to the technical specification should be introduced during the process of local adaptation.

2. SAFETY CONSIDERATIONS

2.1 Risk assessment

Disease security regulations, local health and safety manual, and relevant COSHH legislation must be followed. Only trained, competent and qualified personnel may perform this procedure, as documented in their training records.

FMDV is not a zoonosis, and if all relevant safety procedures are followed then the risk to personnel is reduced to an acceptable level.

Personnel using this SOP are responsible for ensuring that they have read and understood the contents, and the procedure is followed. They must be suitably trained and competent, as documented in their training records. It is the duty of the Head of Department / Group (or nominee) to ensure that staff are aware of this responsibility.

2.2 Safe handling during collection, transport and storage of samples/specimens

FMDV is highly contagious. Samples may potentially be contaminated and must be packaged in accordance with transport of dangerous pathogens legislation.

Compliance with current postal and transport regulations is essential. Specimens should have secure primary and secondary packaging. Appropriate hazard labelling according to local instructions should be followed.

2.3 Safe handling of samples/specimens at the laboratory

All work involving potentially infectious material must be undertaken in buildings which comply with the Specified Animal Pathogens Order (1998) for Group 4 viruses (or equivalent legislation in devolved administrations). Paperwork should be extracted only if safe to do so and before touching sample bottles. Continuous monitoring of the environmental air pressures should be carried out by site engineers. If the pressure falls outside the permitted ranges, the chief technician, or other appointees must be notified. No work handling potentially infectious material may be performed until correct pressure levels are restored.
3. SPECIMEN COLLECTION, TRANSPORT AND STORAGE

3.1 Target tissues and optimal timing of specimen collection

This test requires clotted blood samples, from which serum will be collected on receipt at the laboratory. The timing and selection of animals to be sampled will be determined by the competent authorities and does not form part of this NRM.

3.2 Special considerations to minimise deterioration between collection and processing

Clotted blood samples, or serum, do not require special conditions during transport, but should so far as possible be kept cool and delivered to the laboratory as quickly as possible.

4. TEST PROCEDURE

4.1 Test selection

The method uses a commercial kit, Ceditest® FMDV-NS kit (Cedi Diagnostics B.V., Catalogue No 7610440)

4.2 Reagents

Each kit contains:

- test plates
- negative, weak and positive controls;
- chromogen/substrate solution;
- stop solution;
- demineralised water;
- additive;
- concentrated conjugate, dilution buffer, and washing fluid;
- plate sealer

The kit should be stored at +1 to +8°C observing any expiry date indicated.

In addition, the following are required:

Water: Deionised or glass distilled

Phosphate buffered saline (PBS) (optional, for washing stage – see Section 4.4.2). This may be stored at room temperature until the expiry date indicated on the label of the container.

4.3 Equipment

ELISA reader TiterTek Multiskan Plus MKII or similar with an interference filter of 450nm

Microplate Washer DENLEY Wellwash 5000 or similar

Pipettes Single channel Finnpipettes (or equivalent), variable ranges from 1-10µl, 5-40µl, 40-200µl and 200-1000µl and quality tips. Multichannel Finnpipettes (or equivalent), variable ranges from 5-50µl and 50-300µl and quality tips Multistepper Finnpipettes (or equivalent) variable ranges from 12.5-50µl and 50-250µl and quality tips

Reagent troughs Suitable for multichannel pipetting of a single reagent, any make

Refrigerator or cold room In the range +1°C to +8°C, any make
Freezer In the range -30°C to -5°C, any make

Incubator or hot room Radiant, warm wall incubator in the range +35°C to +39°C, any make

Waterbath Any type in the range +35°C to +39°C. Not essential but useful for rapid warming or thawing of reagents or samples

Class II safety cabinet BS5726, any make

Glassware/plasticware A selection of bottles, flasks, graduated cylinders, graduated pipettes, storage bottles with caps, wash bottles, tubes such as polypropylene centrifuge tubes with lids or eppendorf tubes and suitable racks.

Timer Countdown type with an audible alarm, any make

Absorbent towels/paper Disposable or cloth, lint-free and non-abrasive

Marker pens Waterproof, indelible.

Gloves Disposable (Tru-Touch or Micro-Touch or similar), any make

4.4 Test Procedure

Note: Equilibrate all reagents need to room temperature before use. All glassware and tips used must be clean and sterile. Use fresh tips for each sample or reagent transfer. Prior to the test, mark and number individual microplates to show proposed plate layout. Record on a plate plan.

4.4.1 DAY 1

Prepare day 1 working solutions:

Dilution buffer

Dilute concentrated dilution buffer supplied 1:1 (v/v) in deionized water.

Additive

Reconstitute the lyophilized additive with the demineralised water supplied. Volume as stated on the vial. Gently agitate the vial to dissolve any remaining material. Allow the solution to stand at least 15 minutes at room temperature before use. Unused prepared additive should be stored between -5 and -30°C.

ELISA buffer

Add reconstituted additive to dilution buffer to a final concentration of 10% (v/v). Prepare 24 ml per test plate. Store unused ELISA buffer at +2-8°C.

Remove plates from bags and dispense 80µl of prepared ELISA buffer to all wells.

Dispense 20µl of negative control to well A1 and B1 or to the designated well in duplicate. Dispense 20µl of weak positive control to wells C1 and D1 or to the designated well in duplicate. Dispense 20µl of positive control to well E1 and F1 or to the designated well in duplicate. Dispense 20µl of test serum samples to the designated well in duplicate.

Seal plates using the supplied plate sealer. Shake plates gently and incubate at +20-25°C for 16-18 hours.
4.4.2 DAY 2

**Prepare day 2 working solutions:**

**Washing buffer**

Dilute the concentrated washing fluid 1/200 in deionised water (see label on vial). The kit contains enough concentrated washing fluid to prepare 12 Litres.

*Note:* We have compared washing plates as stated in the manual with washing by completely flooding 3 times with PBS and find no difference.

**Conjugate**

Freshly prepare the working dilution of the conjugate following the instruction on the label of the vial in ELISA buffer.

Empty the well contents into the sink using an abrupt downward hand motion. Wash plates with washing buffer or PBS for three times manually or using plate washer. Slap the inverted plate onto lint free absorbent toweling to remove residual contents.

Dispense 100µl of prepared conjugate to all wells of the plate, seal or cover the plate and incubate at +20-25°C for an hour.

Wash plates as above.

Dispense 100µl chromogen/substrate solution (provided ready for use) in numerical order to all wells of the plate. Incubate for about 20 minutes at +20-25°C.

During the chromogen incubation time, switch on the plate reader and check the appropriate filter (450 nm) is present.

Dispense 100µl of stop solution (provided ready for use) to all wells in the same order as the chromogen/substrate solution was added. Tap the side of the plates to ensure even mixing.

Measure the optical density (OD) of the wells at 450 nm preferable within 15 minutes after color development has been stopped. Blank the spectrophotometer against air then initiate the reading sequence and read all test plates.

4.5 Calculation and interpretation of results

Calculate the mean OD$_{450}$ value of wells A1 and B1 (negative control wells) (= OD max.)

Calculate Percentage Inhibition (PI) value of test samples and controls manually or automatically by the software installed in the computer which connected to the plate reader using the following formula

$$PI = 100 - \left( \frac{OD_{450} \text{ of test or control sample}}{OD_{450} \text{ max.}} \right) \times 100$$

Print out OD$_{450}$ values and percentage inhibition (PI) values (if applicable) of each of all plates. Label printouts appropriately to show plate plan.

Accept the assay if OD$_{450}$ or PI values of controls meet the following criteria

a. The mean OD$_{450}$ of wells A1 and B1 (negative control, OD$_{450}$ max.) must be > 1.000
b. The mean PI value of the weak positive control must be > 50%
c. The mean PI value of the positive control must be > 70%
Calculate the mean PI of each sample and interpretation of test results as following

<table>
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<th>PI value less than 50%</th>
<th>Negative (No antibodies to FMDV 3ABC present)</th>
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<tr>
<td>PI value greater than or equal to 50%</td>
<td>Positive (Antibody to FMDV 3ABC present)</td>
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If the OD of a test sample is higher than the OD$_{450}$ max, the PI of this sample can be interpreted as 0%.

Any laboratory carrying out this test in the UK must consult with Pirbright for advice on the appropriate interpretation criteria. Guidance on the selection and application of FMD serology is also to be found in the OIE Manual for Diagnostics and Vaccines and in the OIE Animal Health Code.

### 4.6 Troubleshooting

If the mean OD of the negative control is below 1.000 possibly the chromogen/substrate solution is too cold. Preheat or incubate the solution to +20-25°C for up to 30 minutes.

If the mean OD of the negative control is above 2.00 a shorter incubation period with the chromogen/substrate solution is recommended.

If the colour which develops is uneven (i.e. there is a large variation between duplicates of individual samples), more care is need with procedures including pipetting, mixing or washing. Cover plates all the time during the incubation or storage to avoid uneven evaporation of fluid.

Should any unexpected failure of the assay occurs, the technical support representatives of Cedi Diagnostics B.V. can be consulted by means of mail, email or telephone as following:

Mail: Cedi-Diagnostics B.V.  
P.O.box 2271  
NL-8203 AG  
Lelystad  
The Netherlands

Tel: ++31 320 238 320  
Fax: ++31 320 214 379  
E-mail: cedidiagnostics@wur.nl  
Website: www.cedi-diagnostics.com

### 4.7 Quality Assurance

All tests must be carried out in an ISO 9001 certified facility, and should if possible be accredited to ISO 17025 standard. External quality assurance (proficiency testing) will be organised by the National Reference Laboratory at Pirbright as appropriate.

### 5. REPORTING PROCEDURE

All valid results must be reported to the competent authorities of the relevant administration. Detailed instructions for reporting will be issued in the event of this NRM being activated at laboratories other than Pirbright.
6. REFERENCES


APPENDIX 1 – REFERENCE LABORATORIES IN THE UK

UK National Reference Laboratory for FMD
Institute for Animal Health
Pirbright Laboratory
Ash Road
Pirbright
Woking
Surrey
GU24 0NF

Tel: 01483 232441
Fax: 01483 232448