COMPARISON OF THREE ELISA KITS FOR THE DETECTION OF ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS NON-STRUCTURAL PROTEINS

WIESław NIEDBALSKI

Department of Foot-and-Mouth Disease, National Veterinary Research Institute, 98-220 Zduńska Wola, Poland
e-mail: wieslaw@piwzp.invar.net.pl

Received for publication December 10, 2004.

Abstract

Three foot-and-mouth disease virus non-structural protein antibody kits: CHEKIT FMD-3ABC, Ceditest FMDV-NS and 3ABC-ELISA were compared. These ELISAs were performed using panel of sera collected from negative (non-infected), vaccinated with trivalent foot and mouth disease vaccine, experimentally infected cattle as well as from animals that had been vaccinated and subsequently infected. Moreover, the panel of the FAO/OIE international reference sera for the purposes of Phase XVIII exercise was tested. The Ceditest kit had a better relative specificity (99.7% for naïve and 98.8% for vaccinated cattle) than the CHEKIT and 3ABC-ELISA kits (97.2 – 98.8% and 98.1 – 99.2%, respectively). A relative sensitivity of all used kits exceeded 98%, however, the Ceditest FMDV-NS kit had the higher sensitivity (99.1%) than the CHEKIT and 3ABC-ELISA (98.2%). The weak 2 O SKR 1/2000 reference serum tested within Phase XVIII collaborative study was detected only in Ceditest kit. It can be concluded that all tested kits can be a useful tool for export/import serological examination, for foot and mouth disease control programmes and especially for eradication campaigns in situation where emergency vaccination was applied.

Key words: cattle, foot and mouth disease, aphthovirus, diagnosis, ELISA.

Foot and mouth disease (FMD) is a highly contagious and devastating disease of all cloven-hoofed animals that still affects extensive areas of the world (26). It is one of the most important economic diseases of livestock owing to both the production losses caused by the disease and disruption caused in international trade with disease-free countries (10). The current method of FMD control in Europe still involves culling of infected and contact animals (“stamping out policy”), restricting animal movement and zoosanitary measures. The ring emergency vaccination is also acceptable, as it was practised during the eradication campaign in the Netherlands in 2001 (1). Recently, the applying of modern FMD vaccines as an alternative method of disease control is widely discussed (17). The new OIE code states that regarding freedom of FMD without vaccination a country may use emergency vaccination, but has to either cull all the vaccinated animals or use serological tests that detect antibodies against foot and mouth disease virus (FMDV) non-structural proteins (NSPs) for the differentiation of infected from vaccinated animals. Moreover, the ability to distinguish these animals is important for export/import serological examination. That is why the considerable efforts should be directed towards the validation of available diagnostic kits for the detection of antibodies to FMDV NSPs. This is needed in order to calculate the sensitivity and specificity requirements for these tests.

In the differentiation procedure either panels of proteins (2, 3, 4) or individual proteins 3D (20, 27), 2C (11), 3AB1 (24) or 3ABC (6, 22) have been used. The response to 3ABC and its cleavage products (mainly 3AB, 3A and 3B) and to 2C has been found to be the most reliable indication of FMDV infection (2, 3, 6, 11, 14). The 3ABC-ELISA has been applied in our laboratory since 1998 (15). Our previous studies on the diagnostic potential of the 3ABC-ELISA showed that this assay can be a useful tool for the differentiation of infected cattle from vaccinated ones (16).

Currently, several commercial ELISAs for FMDV NSPs antibodies are available. In the present study, the specificity and sensitivity of 3 ELISA kits were compared on naïve, infected, vaccinated and on vaccinated and subsequently infected animals.

Material and Methods

Sera. The following sera were examined:

a/ set of naïve sera: a total of 420 samples of sera from Poland which originated from healthy cattle neither vaccinated nor exposed to FMDV as confirmed
by the VNT results. These sera were tested as part of the national serosurveillance programme for FMD (18).

b/ positive sera: 180 "post-vaccination" sera were collected from animals repeatedly vaccinated in 1985-1989 with trivalent FMD vaccine. A total of 49 convalescent sera from animals infected with FMDV were supplied by the World Reference Laboratory for FMD at Pirbright, U.K. and 66 samples of sera collected during the commercial aqueous monovalent A24 vaccine potency test were kindly provided by Dr B. Haas from the Federal Research Centre for Virus Disease of Animals in Tübingen (Germany).

c/ the panel of the FAO/OIE international reference sera generated for the purposes by the FAO Collaborative Studies Phase XVIII: a strong positive, weak positive 1 and weak positive 2 against FMDV O SKR 1/2000 serotype (21).

ELISA. The serum samples were examined with 3 ELISA kits: CHEKIT FMD-3ABC (Bommeli Diagnostics, Switzerland), Ceditest FMDV-NS (Cedi-Diagnostics, B.V. the Netherlands) and 3ABC-ELISA (Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna - IZSLER, Brescia, Italy). These assays are the indirect ELISAs, based on the detection of antibodies to the FMDV 3ABC polyprotein. The 3ABC-ELISA was performed according to the method developed by De Diego et al. (6). A positive reaction was scored when the OD492 values, obtained after subtraction of the background of wells without antigen, were higher than 0.2 (cut-off level). Reagents were kindly provided by Dr E. Brocchi from the IZSLER. The CHEKIT FMD-3ABC and Ceditest FMDV-NS were used according to the manufacturers’ instructions.

Sensitivity of ELISA kits. The relative sensitivity of the ELISA kits was evaluated by examining serum samples collected from cattle following experimental infection with the different serotypes of FMDV as well as from cattle vaccinated with a commercial aqueous monovalent A24 vaccine and challenged 28 days post vaccination with homologous FMDV.

Specificity of ELISA kits. Comparative specificity of ELISA kits was estimated using negative sera originated from healthy cattle and collected from vaccinated cattle.

Results

The results of relative sensitivity of the ELISA kits are presented in Table 1. When testing by the CHEKIT and 3ABC-ELISA, 112 of the 114 samples of sera scored positive (98.2%). The sensitivity of Ceditest FMDV-NS based on 115 positive animals was 99.1%.

The results of comparative specificity the ELISA kits are shown in Table 2.

Table 1
Comparison of relative sensitivity of used ELISA kits

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of sera examined</th>
<th>Number of positive sera</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEKIT FMD-3ABC</td>
<td>114</td>
<td>112</td>
<td>98.2</td>
</tr>
<tr>
<td>Ceditest FMDV-NS</td>
<td>115</td>
<td>114</td>
<td>99.1</td>
</tr>
<tr>
<td>3ABC-ELISA</td>
<td>114</td>
<td>112</td>
<td>98.2</td>
</tr>
</tbody>
</table>

Table 2
Comparative specificity of the ELISA kits

<table>
<thead>
<tr>
<th></th>
<th>CHEKIT FMD-3ABC</th>
<th>Ceditest FMDV-NS</th>
<th>3ABC-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Naïve animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative results</td>
<td>411</td>
<td>402</td>
<td>398</td>
</tr>
<tr>
<td>Positive results</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>98.8</td>
<td>99.7</td>
<td>99.2</td>
</tr>
<tr>
<td>(B) Vaccinated cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative results</td>
<td>178</td>
<td>179</td>
<td>164</td>
</tr>
<tr>
<td>Positive results</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>97.2</td>
<td>98.8</td>
<td>98.1</td>
</tr>
</tbody>
</table>
A total of 411 serum samples collected from naïve animals were tested. Among them, 406 were negative in CHEKIT FMD-3ABC (98.8%). Of 402 sera tested by Ceditest FMDV-NS, 401 were negative (99.7%) and when testing 398 sera with 3ABC-ELISA, 394 gave positive reaction (98.1%). When testing 178 samples of sera collected from vaccinated cattle, 173 scored negative in CHEKIT test (97.2%). Out of 179 sera examined in Ceditest, 177 gave positive reaction (98.8%) and when testing 164 samples in 3ABC-ELISA, 161 were negative (98.1%).

The results of the examination of international reference sera from FAO/OIE Phase XVIII Collaboration Exercise are set out in Table 3.

All the reference sera collected 34 days after an experimentally-induced infection were positive only in CHEKIT FMD-3ABC ELISA. In the other used ELISAs the O weak 2 serum gave negative results.

<table>
<thead>
<tr>
<th>Test</th>
<th>Phase XVIII reference sera against FMDV O SKR 1/2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O strong</td>
</tr>
<tr>
<td>CHEKIT FMD-3ABC</td>
<td>90 PIa (positive)</td>
</tr>
<tr>
<td>Ceditest FMDV-NS</td>
<td>94 PIa (positive)</td>
</tr>
<tr>
<td>3ABC-ELISA</td>
<td>0.82b (positive)</td>
</tr>
</tbody>
</table>

a – percentage of inhibition (PI)

b – OD492

Discussion

The aim of this study was to determine diagnostic value of 3 FMDV NSPs kits: the FMD-CHEKIT-ELISA from Bommeli (23), Ceditest FMDV-NS based on the method of Sorensen et al. (25) and 3ABC-ELISA developed by De Diego et al. (6). These assays are easy and quick to perform, especially the CHEKIT and Ceditest which employ a recombinant 3ABC antigen directly coated to the ELISA plate. Using the same set of cattle sera, we compared all the kits for their sensitivity and specificity. We found a comparable sensitivity and high specificity of all used ELISAs. However, the Ceditest had the relatively higher specificity, which is similar to its performance reported elsewhere (7, 9). The comparable sensitivity and specificity of commercially available NSPs kits performed recently by Moonen et al. (13) showed that these validation levels can differ considerably depending on the time after experimental inoculation of animals. In our experiment, most of the tested sera were collected 21-140 d after infection.

There are no internationally agreed reference standard sera for the calibration of NSP-based test methods. However, the panel of the FAO/OIE international reference sera generated for the purposes of the FAO Collaborative Studies Phase XVIII was recommended to detect antibodies to the non-structural proteins of FMDV (21). The results obtained in our laboratory and by the most of participating laboratories confirmed the highest sensitivity of Ceditest kit. It was found that the type O SKR reference sera would be useful as NSP reference sera, since the strong and weak 1 positive sera were consistently detected, whereas the weak 2 serum was more borderline (21).

In May 2002, the OIE approved a new set of guidelines that expands on the chapter in the OIE Diagnostic Manual and the paper of Jacobson (8). The stages of assay development and validation are described in details and examination of these reveals a number of critical issues for NSP assays validation. In order to calculate the sensitivity and specificity requirements of any diagnostic tests it is important to know the purpose of testing and the expected prevalence of infection to be detected. However, for FMDV NSPs test, there is a lack of information on the expected within-herd prevalence of persistently infected animals in post-vaccination populations. Consequently, requirements for sensitivity and specificity have not been accurately determined. Validation of NSP tests is one of the tasks of the EU research project FMD-ImproCon (SSPE-CT-2003-503603). So far about 19 000 tests have been performed in 11 laboratories using 6 NSP assays. A preliminary analysis was made to compare the diagnostic sensitivity and specificity of the tests in several animal categories at different time points after vaccination and/or infection. These kits are to some extent validated but they lack information on performance on species in which overt symptoms may be mild or absent altogether, such as sheep. Therefore these investigations will be continued on comparison of different methods on sera from sheep, goats and pigs (5).

The results of our comparative study have shown that the applied NSP ELISA kits, due to their
high specificity and sensitivity, can be a useful tool in FMD control in a herd and determination of virus circulation in vaccinated populations. The test for NSP antibodies in connection with tests for the detection of antibodies to FMDV structural proteins (19) should be used in sero-surveillance of livestock following vaccination during or after an outbreak in otherwise FMD-free countries.

References
