

## ***The use of a 3ABC marker-test for FMD***

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### **Introduction**

Vaccination plays an important role in the control of FMD in Asia, Middle East, Africa and South America. In most FMD-free countries a non-vaccination policy is in place. Recent outbreaks in Europe clearly demonstrated the risk of this policy. Using conventional diagnostic techniques, up to now it was not possible to distinguish FMD infected animals from purely vaccinated animals. In vaccinated areas disease control authorities had limited possibilities to monitor virus presence or circulation.

### **Vaccine composition**

Modern, state of the art-vaccines are based upon highly purified antigens, which are free from Non-Structural Proteins (NSP) of the FMD virus. Other vaccines may be partly purified and contain a reduced amount of NSP. Animals, vaccinated with highly purified, NSP-free vaccines, produce antibodies against the Structural Proteins (SP) but not against NSP.

FMD virus infection induces antibodies against both SP as well as NSP.

NSP-free or NSP-reduced vaccines in combination with a NSP-test lead to a so-called marker-system.

### **The FMD marker principle**

A test, which differentiates antibodies due to vaccination from antibodies due to infection, would be of great value in FMD control. Several tests, which are based on non-structural proteins (NSP) have been described (Berger et al., 1990, Neitzert et al., 1991, Bergmann et al., 1993, Lubroth et al., 1995).

The success of the principle has also been demonstrated for pigs (Rodriguez et al. 1994).

For the screening of large numbers of samples an ELISA would be highly preferable. An indirect-trapping ELISA for the detection of antibodies against 3ABC has been reported (De Diego et al., 1997). The sensitivity of the assay on experimental sera post-infection was reported to be 100%. The specificity was reported to be more than 99%.

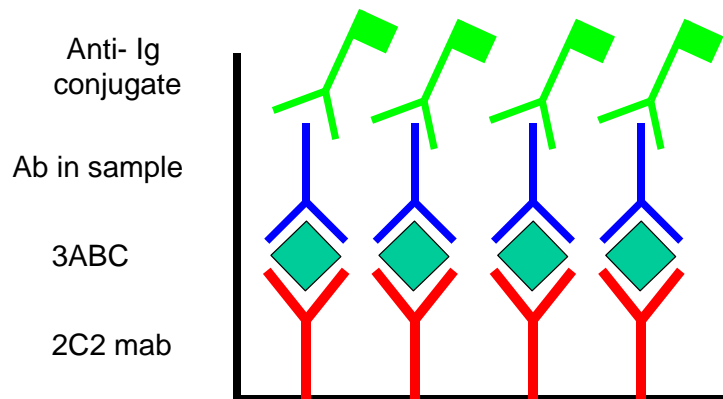
From 1994 to 1997 the Commission of the European Communities sponsored a research program, Concerted Action CT93 0909, on the potential use of tests, based on NSP, for the differentiation between antibodies, induced by vaccination, from antibodies, induced by infection. The conclusions are reported in the Proceedings (Proceedings, 1998). It is concluded, that the polyprotein 3ABC is the single most reliable indicator of infection.

Bommeli Diagnostics, Switzerland, subsidiary of Intervet International, has developed Chekit-FMD-3ABC, an ELISA testkit, in collaboration with the World Reference Laboratory in Pirbright (U.K.) and the Istituto Zooprofilattico in Brescia (Italy). The test has been validated in three national reference laboratories.

### Test development

The original test of the World Reference Laboratory in Pirbright (U.K.) and the Istituto Zooprofilattico in Brescia (Italy) was a trapping, indirect ELISA. The test was not ready for use and several sensitive steps had to be performed before the test could actually start. Because of this there have been problems with variable results in several laboratories with the NSP-test.

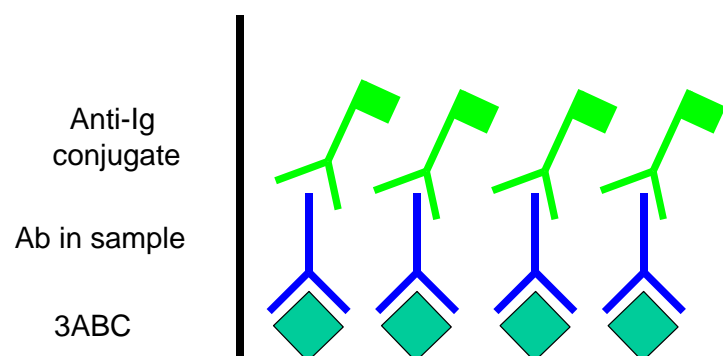
#### **ORIGINAL IAH/IZP 3ABC ELISA (trapping indirect ELISA)**



The new test, Chekit-FMD-3ABC, is a direct ELISA. The test-plates are coated with 3ABC. The test is ready to use. Compared to the original concept this has considerable advantages:

- Test is based on a validated and established system.
- The test is ready to use.
- Monophasic assay.
- Established large scale production.
- Expertise from IAH & IZP for the 3ABC-ELISA system.

#### **CHEKIT-FMD-3ABC**



### **The marker-test principle**

The conventional tests (VN, VP<sub>1</sub>-ELISA) demonstrate antibodies against FMD without differentiation between infection and vaccination. The VN-test has to be carried out in high-containment, because it is carried out with FDM-virus. Both tests are serotype specific. The test will only give a positive result, if the field strain is of the same serotype as the test-strain.

Chekit-FMD-3ABC only demonstrates antibodies after infection and not after vaccination. Chekit-FMD-3ABC is not serotype specific and will be positive for all FMD viruses, irrespective of the serotype.

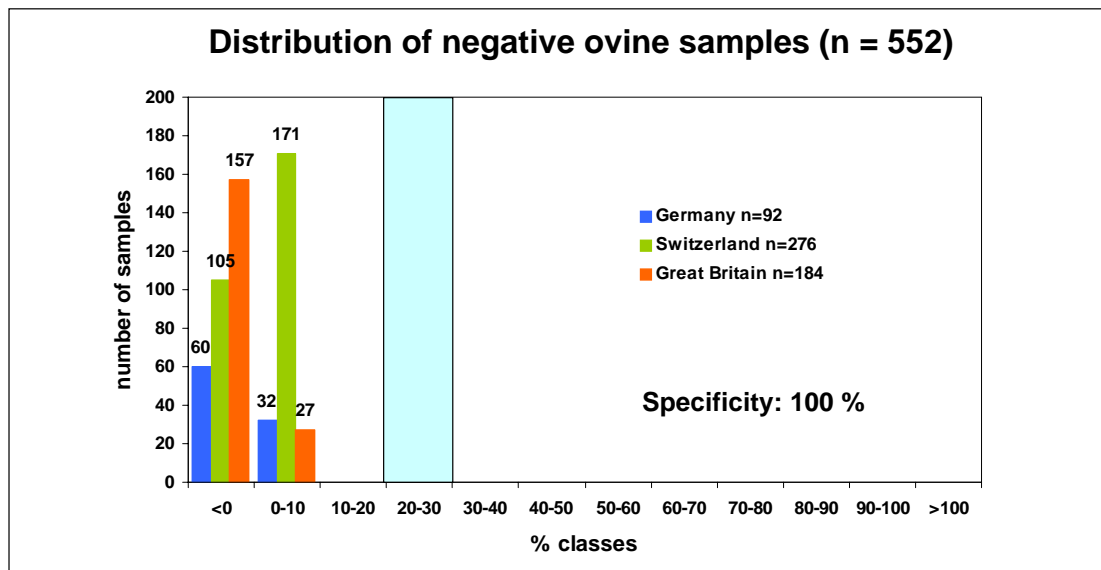
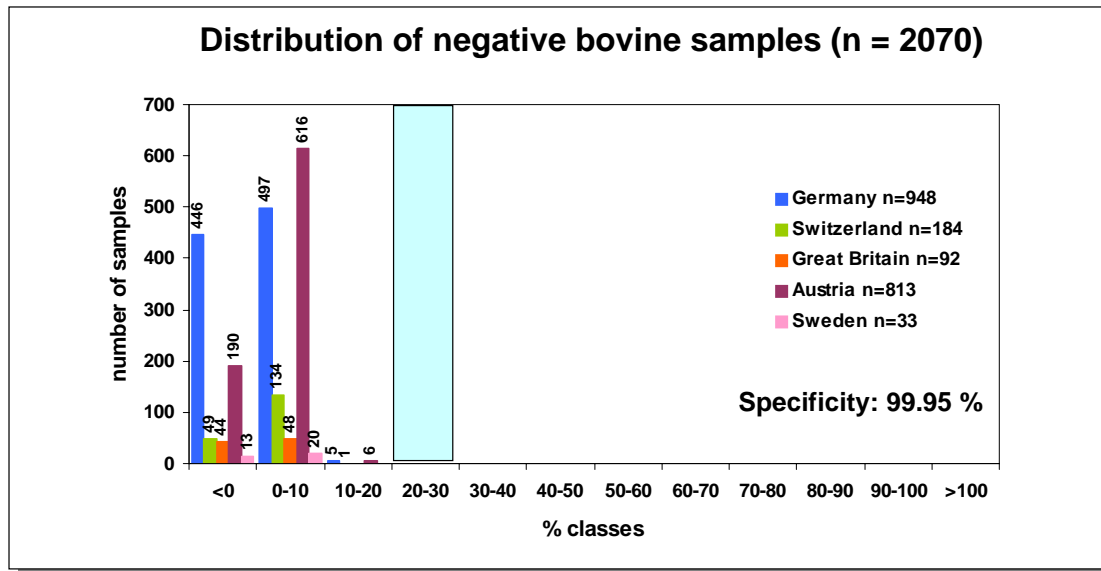
### **Comparison serological results between the conventional and 3ABC marker-test:**

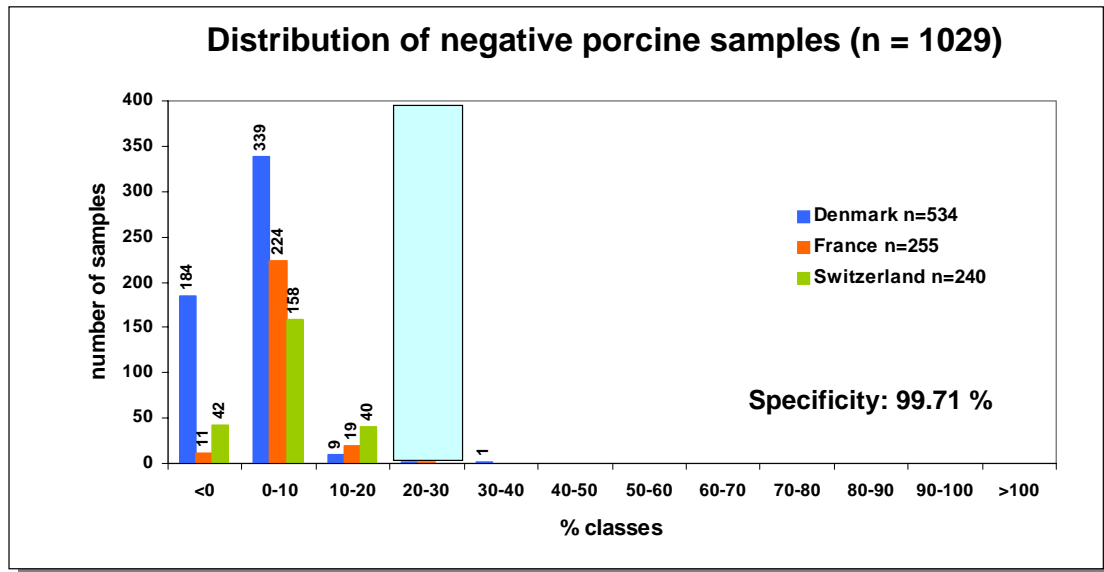
		<b>Results serology</b>	
		Conventional test	3ABC-test
Vaccinated	Infected	+	+
Vaccinated	Not infected	+	-
Not vaccinated	Infected	+	+
Not vaccinated	Not infected	-	-

### **Validation of the test**

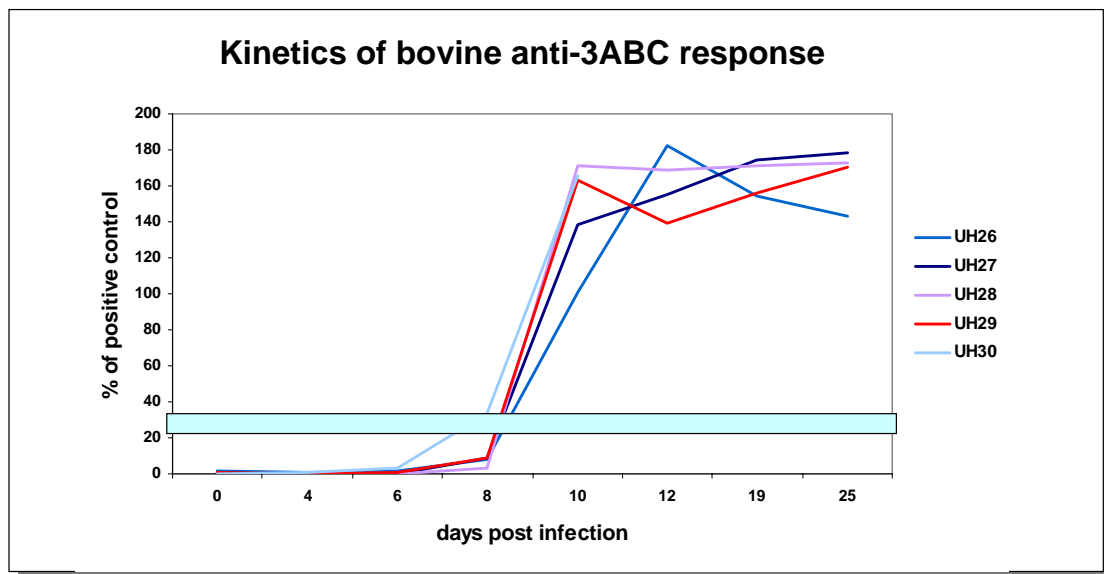
Chekit-FMD-3ABC has been validated with the sera, which have been used for the validation of the original Brescia and Pirbright tests. The sera were from experiments as well as from the field, from well over 5.000 animals, cattle, sheep and swine. The sera involved all main FMDV-types. The sera have been supplied by the Istituto Zooprofilattica in Brescia (Italy), World Reference Laboratory Pirbright and the ID-Lelystad Institute from The Netherlands.

The following selected data give an impression on the performance of the testkit in negative and positive animals:

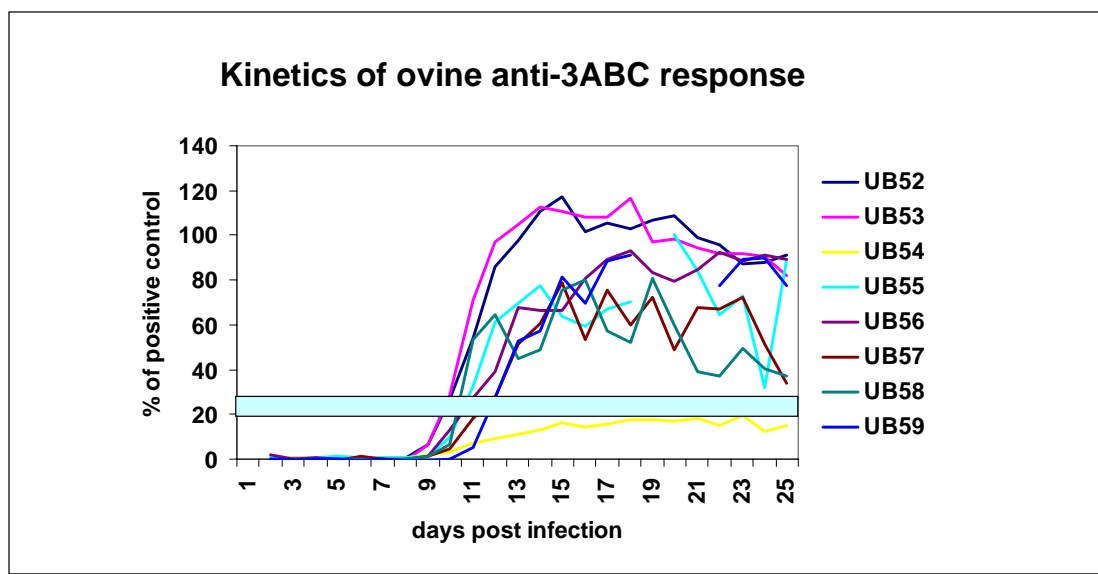




**Antibodies against 3ABC after challenge in cattle:**



### Antibodies against 3ABC after challenge in sheep:



The specificity of sera from vaccinated animals is shown in Table I-III. Vaccination elicits virus neutralizing antibodies in cattle (Table I), in sheep (Table II) and in pigs (Table III). In contrast, none of these sera contain detectable amounts of anti-3ABC antibodies.

Table I. Testing of bovine vaccinated animals<sup>a)</sup>

	Group 1				Group 2			
	ELISA (%) <sup>b)</sup>		VNT (log <sub>10</sub> <sup>c)</sup>		ELISA (%)		VNT (log <sub>10</sub> )	
pv weeks	x	SD	X	SD	x	SD	x	SD
0	-0.7	2.65	<0.30	NA	-2.7	0.60	<0.30	NA
1	-0.5	3.69	0.53	0.35	-2.1	0.68	<0.30	NA
2	-2.8	0.44	1.28	0.11	-2.8	0.24	<0.30	NA
3	-2.6	1.32	0.9	NA	-1.3	1.40	0.3	NA
4	-1.8	0.08	0.83	0.11	-1.0	0.84	0.6	NA
5	2.1	3.73	>2.55	NA	-2.4	0.20	1.65	NA

- a) Cattle (n=2), were immunized with O<sub>1</sub> Manisa (Group 1) or served as control (Group 2) Both Groups were challenged with the homologous strain.  
 b) CHEKIT-FMD-3ABC  
 c) Strain: O<sub>1</sub> Manisa  
 NA: not applicable

Table II. Testing of ovine vaccinated animals<sup>a)</sup>

pv weeks	ELISA (%) <sup>b)</sup>		VNT (log10)					
	X	SD	A <sub>22</sub> Iraq		O <sub>1</sub> Manisa		Asia <sub>1</sub> Shamir	
			X	SD	X	SD	x	SD
0	-2.3	0.53	<0.30	NA	<0.30	NA	<0.30	NA
1	-2.5	0.72	<0.30	NA	0.75	0.28	0.99	0.29
2	-2.2	0.41	0.63	0.33	1.29	0.59	1.98	0.27
3	-2.5	0.28	0.57	0.22	1.53	0.61	1.62	0.27

- a) Sheep (n=5), were immunized with a vaccine comprising strains A<sub>22</sub> Iraq, O<sub>1</sub> Manisa, O<sub>1</sub> Marocco, and Asia<sub>1</sub> Shamir  
b) CHEKIT-FMD-3ABC  
NA: not applicable

Table III. Testing of porcine vaccinated animals<sup>a)</sup>

pv weeks	ELISA (%) <sup>b)</sup>		VNT (log10)					
	X	SD	A <sub>24</sub> Cruzeiro		O <sub>1</sub> Manisa		C <sub>1</sub> Detmold	
			X	SD	X	SD	x	SD
0	5.2	2.69	0.09	0.20	0.09	0.20	0.09	0.20
1	7.8	1.42	0.42	0.27	0.99	0.39	0.45	0.50
2	5.2	4.78	0.60	0.21	1.23	0.33	0.84	0.23
3	5.0	1.19	1.11	0.38	1.80	0.35	1.35	0.32
4	4.4	1.92	1.44	0.29	2.01	0.39	1.74	0.31

- a) Pigs (n=5), were immunized with a vaccine comprising strains A<sub>24</sub> Cruzeiro, O<sub>1</sub> Manisa, and C<sub>1</sub> Detmold  
b) CHEKIT-FMD-3ABC  
NA; not applicable

### **Testing strategies**

FMD disease control measures, whether used for control, eradication or prevention, have to be adapted to local requirements. It is therefore impossible to give precise recommendations on the practical use of the test in a specific program. Below are possibilities for use of the 3ABC test and marker-system, which may function as a starting point for the development of the right control strategy.

Whilst setting the strategy for the test it is important to reckon with the main characteristics of Chekit-FMD-3ABC:

- The test is serotype aspecific.
- Antibodies against 3ABC will be demonstrated as from 10-14 days after infection.
- The test does not contain any infectious material and can be run in every laboratory, which is equipped for ELISA.
- The test provides results within hours.

## **1. General screening**

Chekit-FMD-3ABC has considerable advantages, also in situations where vaccination is not being practised. The test detects antibodies against the 3ABC polyprotein due to infection. The 3ABC protein is not serotype specific. A positive result of Chekit-FMD-3ABC indicates an infection with the FMD virus, irrespective of the serotype.

### **1.1. Screening border control**

The 3ABC test is an ideal test for border control. Independently of which FMD virus serotype is circulating, Chekit-FMD-3ABC allows the detection of serologically positive animals. The results are known within hours, which prevents unnecessary waiting times before cross-border traffic.

### **1.2. Screening suspected infections**

Chekit-FMD-3ABC will confirm the diagnosis FMD within hours. In case of a positive result further work is necessary to establish the serotype. However zoosanitary measures can be taken before the serotype is established.

## **2. Marker-system: Import / border control**

### **2.1. Border control**

International trade intrinsically carries the risk on spread of diseases. Since vaccination may be practised in some areas the use of a marker test provides authorities with the real information on infection.

Although the sensitivity is more than 99%, there is a small risk that individual animals may be missed. Therefore it is recommended use Chekit-FMD-3ABC as a herd-check and to test all animals at the border.

### **2.2. Export**

Breeding farms and artificial insemination centres may decide (subject to Government approval) to opt for FMD vaccination, even if the country of origin is free from FMD. Through vaccination animals can be protected in countries, which have the risk on incidental outbreaks. Because of international traffic, the risk is realistic in most countries. In addition, in case of export of animals to FMD endemic areas it is highly attractive to vaccinate the animals well before the transport in order to deliver animals, which have full protection at the moment of arrival in the importing country.

## **3. Marker-system: Endemic situations:**

### **3.1. Farm-use:**

In countries, where FMD is endemic, individual farms (e.g. high standard farms, breeding farms, A.I. centres, experimental stations), may decide to aim for FMD status with vaccination.

Special attention needs to be given as to a testing scheme and sample size.

### **3.2. Regional use:**

Although endemic, FMD virus may be circulating in livestock-intensive areas. Usually in less intensive areas virus circulation is less and it may be possible to aim for regional FMD eradication. Initially vaccination can be intensified in the region and if the number of 3ABC positive animals is close to zero, eradication may be continued without vaccination.

A FMD regional eradication is important as it may provide a source of FMD-free animals for restocking on depopulated farms.

### 3.3. National use:

In FMD endemic countries, a conventional VP<sub>1</sub> Elisa or VN test may be used to check for vaccination discipline. The 3ABC marker test provides information on the frequency of FMD infection in the same (vaccinated) animals and additional information on vaccination discipline. Animals, which show high titres in a conventional test are frequently considered to be well-vaccinated. With Chekit-FMD-3ABC it can be decided, whether high VN-titres are due to efficient vaccination or due to a field infection.

In the past the status of “FMD free with vaccination” was obtained, based on absence of clinical disease and absence of FMD infection in sentinel animals. Some FMD strains show very few clinical symptoms. The use of sentinel animals is therefore not very reliable. Besides, an infection of sentinel animals may well hamper earlier results in reduction of virus circulation.

The 3ABC test is a reliable herd-test to trace infected farms. While using Chekit-FMD-3ABC, the use of sentinel animals is no longer a prerequisite to trace virus circulation.

If the status “FMD free with vaccination” has been obtained with the use of a 3ABC marker test, the absence of circulating virus is sure and the country may safely take the necessary steps to stop vaccination.

## **4. Outbreaks in formerly FMD free countries / regions**

Most FMD-free countries adopted the non-vaccination policy. The vulnerability of the livestock industry (under the non-vaccination policy) became clear in the recent outbreaks in Europe.

In case of an outbreak in a (formerly) FMD-free country, vaccination remains an important tool in FMD-control. This has a.o. been demonstrated in The Netherlands, where vaccination has been used in combination with a very stringent stamping-out policy. Within 2 weeks after vaccination, there were no new outbreaks of FMD.

Vaccination however was only allowed on the condition, that all vaccinated animals would be identified and killed and destroyed within 2 months.

Because of the possibility to trace infection in vaccinated animals, Chekit-FMD-3ABC enables authorities to start emergency vaccination whilst monitoring the spread of the virus. Vaccination prevents the spread of the disease. Because infection can be traced in vaccinated animals, it is no longer necessary to slaughter all vaccinated animals. Massive (preventive) culling procedures have met strong criticism from the public because of environmental, ethical and emotional reasons and are economically unattractive.

Vaccinated animals from farms without 3ABC positive animals can safely be offered for human consumption and will not pose a risk on spreading of FMD. If considered necessary, it is still possible to restrict slaughter of vaccinated animals from 3ABC negative farms to slaughterhouses within the vaccinated area.

With the 3ABC marker-test and –vaccine authorities have the full choice to decide for protective or suppressive vaccination in one of the following geographical scenario's:

- Ring vaccination around infected herds: prevention of spread from infected farms to neighbouring farms.
- “Fire-wall vaccination”: if there is a risk of spread of infection from infected regions to FMD-free areas, it may be worthwhile to vaccinate a corridor between

both areas. With the 3 ABC marker-test it is still possible to monitor spread of infection within the vaccinated area.

- Regional vaccination: if FMD is established in a certain area and the rest of the country is still free, it may be decided to vaccinate all susceptible animals in the region. The use of the 3ABC test still enables authorities to monitor the spread of disease. Slaughter and consumption of vaccinated animals (provided they are from 3ABC-negative farms) is still possible without the risk on further spread.

Last but not least, success in FMD control depends on many control mechanisms, of which vaccination and testing is only one. Stringent zoosanitary measures remain essential to success.

### **Practical issues of Chekit-FMD-3ABC**

- The 3ABC polyprotein is concluded to be the single most reliable indicator of infection (Concerted Action CT93 0909, 1998).
- The 3ABC protein is not serotype specific. A positive result of Chekit-FMD-3ABC indicates an infection with the FMD virus, irrespective of the serotype.
- Chekit-FMD-3ABC does not contain any infectious material and therefore does not, contrary to a VN test, have to be performed in high containment.
- In case of an outbreak large numbers of samples can be processed in a standard laboratory in a robotted system.
- Waiting time for the test is minimal, since Chekit-FMD-3ABC provides results within hours.
- Chekit-FMD-3ABC, developed by Bommeli Diagnostics, has been validated and the tested sera showed, that both specificity and sensitivity are higher than 99%. These figures need to be confirmed on larger numbers of known-positive samples. The 3ABC marker-test is recommended for herd diagnosis rather than for individual diagnosis.
- There are 2 versions of the test: bo-ov for cattle and sheep, containing a ruminant conjugate, and pc for swine, containing porcine conjugate.
- Chekit-FMD-3ABC is the first commercially available 3ABC testkit and is produced under ISO 9001 certified conditions.

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