# Gamma Interferon Assay for Cellular Immune Response in Cattle Vaccinated With FMD Vaccine Adjuvanted with Different Montanide Oils

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## Abstract

Cell-mediated immunity is critical for the prevention and control of Foot and Mouth Disease (FMD). Despite significant advancements in modern vaccinology, inactivated whole virus vaccines for FMD remain the mainstay for prophylactic and emergency uses. Emergency vaccination as part of the control strategies against foot-and-mouth disease virus (FMDV) has the potential to limit virus spread and reduce large-scale culling. Many efforts are currently devoted to improve the immune responses and protective efficacy of these vaccines. Adjuvants, which are often used to potentiate immune responses, provide an excellent mean to improve the efficacy of FMD vaccines.Aim: To evaluate three oil adjuvants namely: Montanide ISA-206, ISA-201 and ISA-61 for adjuvant potential in inactivated FMD vaccine by determination of the produced amounts of interferon-gamma (IFN-gamma) in cattle vaccinated with FMD trivalent vaccine adjuvanted with different Montanide oils using interferon-gamma Assay for evaluation of FMD virus-specific cell-mediated immunity.

***Index terms—***

## 1 I. Introduction

oot-and-mouth disease virus (FMDV) causes footand-mouth disease (FMD), a contagious and fatal disease in cloven-hoofed animals, characterized by vesicles in the mouth, tongue, hoofs, and nipples and increase in body temperature and appetite loss Depa et al., (2012). the natural route of infection is via the upper respiratory tract or through ingestion of the virus. Initial virus replication usually occurs in the pharyngeal epithelium resulting in primary vesicles Alexandersen and Mowat (2005). Fever and viraemia can occur within 1-2 days resulting in virus excretion from the respiratory tract, faeces, urine, saliva, milk and semen. Virus entering the blood disseminates to various predilection sites such as the mouth and nose, hooves and also sometimes teats and udder, in which secondary vesicles occur, and from which further virus is released Grubman (2005) and Diaz-San et al., **??**2009). The progress in FMD vaccine production was primarily directed towards safety of the vaccine, purity of the antigen, selection of proper adjuvant and endurance of immunity Osama **??**1992). Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humoral or cell mediated immunity Lombard et al., (2007) and Cao (2014). Currently, the double oil emulsion vaccines are preferred for FMD prevention as they can be used to protect all susceptible species, particularly dur-ing an outbreak situation Cox and Barnett (2009). Also, the oil adjuvant vaccines generate higher and long lasting immune responses, and show less inter-ference from maternal antibodies than the aqueous vaccines Selim et al., (2010). In particular, the Montanide TM ISA series of oil-adjuvants (SEPPIC France) have shown superior efficacy for inactivated FMD vaccines in different susceptible animal species Iyer et al., (2000). Recently, SEPPIC has developed a new adjuvants (Montanide ISA-201 and Montanide ISA-61) and claim that those adjuvants induce better immune responses (particularly CMI responses) Seppic. Montanide ISA 201 VG-ready to use oil adjuvant for veterinary

vaccines and Sébastien et al., **??**2013). The ability to stimulate cell-mediated immunity (CMI) and conse-quent inhibition of subclinical infection in ruminants or otherwise induction of sterile immunity is usually insufficient Moonen et al., (2004) Interferons belong to cytokines. They are glycoproteins with multifaceted signal effects on cellular functions among which the antiviral effects belong to the early and non-specific defense mechanisms of organisms against infections Vilcek and Sen (1996).

Interferons (IFNs) are the first line of the host innate immune defense against important, derives from its immunostimulatory and immunomodulatory effects Samuel (2001) and Delcenserie et al., (2008). The assay system has proven to be a rapid, sensitive and inexpensive method for measuring antigen specific cellmediated reactivity when compared with the more traditional lymphocyte proliferation assay. The IFNgamma assay is the first in-vitro cellular assay to be used as a routine diagnostic test in veterinary medicine Rothel et al., (1992). The production of interferongamma by stimulated helper T lymphocytes regulates production of immunoglobulin in vaccinated animals Green et al., (2015). IFN-gamma is a modulator of T-cell growth and functional differentiation. It is a growthpromoting factor for T-lymphocytes and potentiates the response of these cells to mitogens or growth factors. The production of IFN-gamma or IL-4 by subsets of helper T lymphocytes reciprocally regulates production of lgG2a and IgG1.The minimum detectable dose of IFN-gamma is typically less than 5 pg/ml Cubillos et al., (2008) and Bucafusco et al., (2015), while the protective level is more than 38% Sample to Positive (SP %) Gurung et al., (2014).

It has been suggested that cell-mediated immunity may be involved in the clearance viral infection so the importance of Interferon-Gamma (IFN-gamma) in the immune system stems in part from its ability to inhibit viral replication directly, but, most of persistent virus Ilott et al., (1997) and Childerstone et al., (1999) and it has been hypothesised that the initiation of FMDV persistence is correlated with the amount of interferon produced in the cells Phillips and Dinter (1963). FMDV strains modified by passage in alternate hosts or repeated passage in cell cultures have reduced virulence in cattle and, in contrast to more virulent wildvirus, will induce the production of interferon Zhang et al., (2014) with a correlation between lack of virulence in cattle and increased IFN production Alexandersen et al.,(2002).

The present work aims to evaluate the FMD virus-specific cell-mediated immunity in cattle vaccinated with FMD vaccine adjuvanted with different Montanide oils using interferon-gamma Assay, in order to determine to any extent FMD trivalent vaccine is able to elicit a sterile immunity.

# 2 II. Material and Methods

# 3 a) Cell and virus

Baby Hamster Kidney cell line (BHK21) Clone 13 maintained in FMD Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo according to the technique described by Macpherson and Stocher (1962) Killington et al., (1996). The viral suspension was concentrated at 25,000 rpm, for 5 hours at 4? in a highspeed centrifuge (Avanti J25, Beckman Coulter, and Fullerton, CA, USA), the virus in the bottom was removed and polled. The virus was further concentrated in ultracentrifuge 35,000 rpm/min, 3 hours at 4?, the viral pelted polled and aliquots of the concentrated virus preserved at -80?.

# 4 c) FMD viruses inactivation

The concentrated virus stock completely inactivated using Binary Ethyleneimine (BEI) according to Bahnemann (1975), 1%M BEI in 0.2N NaOH was added to the virus suspension to give final concentration of 0.001M of BEI. The virus and BEI mixture were mixed well and the pH adjusted to 8.0 by sodium bicarbonate. The virus was placed in the incubator at 37 o C for 24 hours for inactivation to occur. Sodium thiosulphate was added to give a final concentration of 2% to neutralize the BEI action. The killed vaccine kept at -80?, to use in preparation of vaccine formulation with different Montanide Oil adjuvants (ISA 206,201 and 61) for animal immunization according to FAO. (2012).

# 5 d) Montanide ISA 206

This is a mineral oil based adjuvant which has been developed for the manufacture of Water-in-Oil-in-Water (W/O/W) emulsions mixed with antigen 50% w/w. It was obtained from Seppic, Paris, France.

# 6 e) Montanide ISA 201

This is a mineral oil based adjuvant that has been developed for the manufacture of Water-in-Oil-in-Water (W/O/W) emulsions mixed with antigen 50% w/w. It was obtained from Seppic, Paris, France.

# 7 f) Montanide ISA 61

This is a mineral oil based adjuvant that has been developed for the manufacture of water-in-oil (W/O) emulsions mixed with antigen 60% w/w. It was obtained from Seppic, Paris, France.

# 8 g) Trivalent FMD vaccines preparation i. FMD oil adjuvanted vaccine formulated with Montanide ISA 206

Formulation with oil phase carried out according to the method described by Wael et al., **??**2014), where the oil phase consisted of Montnide ISA 206 mixed with the inactivated viruses as equal parts of an aqueous and oil phase (50% w/ w) and mixed thoroughly.

# 9 ii. FMD oil adjuvanted vaccine formulated with

Montanide ISA 201 Formulation with oil phase carried out according to the method described by Dar et al., (2013) and **??**hab et al., (2015) where the oil phase consisted of Montnide ISA 201 mixed with the inactivated viruses as equal parts of an aqueous and oil phase (50% w/ w) and mixed thoroughly.

# 10 iii. FMD oil adjuvanted vaccine formulated with

Montanide ISA 61 Formulation with oil phase carried out according to the method described by Gurung et al., (2014) where the oil phase consisted of Montnide ISA 61 mixed with the inactivated viruses as 60% of an aqueous and oil phase (60% w/w) and mixed thoroughly.

# 11 h) Animal groups

.Twelve calves (local breed) were clinically healthy and free from antibodies against FMD virus as proved by using SNT and ELISA were used in this study.

Calves used in experimental vaccination were classified into four groups:

# 12 i) Samples collection

Blood samples were collected on 3 rd post vaccination every three days for 2 weeks and later every week up to 10 weeks. Serum samples were collected weekly post vaccination for one month then every 2 weeks post-vaccination till the end of experiment. The immune response was evaluated through the detection of INF-gamma and humoral immune level using Bovine IFN-gamma ELISA assays, SNT and ELISA.

i. Detection of interferon gamma (IFN-gamma) using Bovine IFN-gamma ELISA kits It was applied according to Barnett et al., (2004). The cytokine IFN-gamma was measured in plasma samples from all cattle groups at various time points before and following vaccination using Bovine IFN-? ELISA kit (Mabtech-Sweden -code/3115-1H-20). High protein binding ELISA plates were coated with mAb bIFN-?-1 diluted to 2µg/ml in PBS, PH 7.4, by adding 100 µl/well incubated overnight 4-8?C according to the manual technique. The plates were washed with PBS (200µl/well) before blocking with PBS containing 1% bovine serum albumin for 30 min at room temperature. Blocked plates then were washed five times with PBS containing 0.05% Tween 20(Incubation buffer).

Bovine IFN-gamma was prepared standard by reconstituting content of vial in 1ml PBS to give concentration of 0.5µg/ml and leaved at room temperature for 15 minutes, then vortex the tube and spin down and use immediately. Samples or standards diluted in incubation buffer added as 100µl/well and incubated for 2 hours at room temperature, then washed as before. Then 100µl/well of mAb PAN-biotin at 0.1g/ml in incubation buffer was added, incubated for 1 hour at room temperature and then washed as washing step. Then 100µl/well of Streptavidin-Horse Radish Peroxidase (Streptavidin-HRP) diluted 1:1000 in incubation buffer was added and incubated at room temperature for 1 hour.

Appropriate substrate solution was added as 100µl/well. Finally measured the optical density in an ELISA reader after suitable developing time, absorbance values were read at 492 nm and the results were calculated according to kits typical data. Optical density values were normalized across plates using the following calculation: ii

# 13 . Serum neutralization test (SNT)

The test was performed by the microtechnique as described by Ferreira (1976) in flat bottom tissue culture microtitre plates.

iii. Enzyme linked immunosrobent assay (ELISA) It was carried out according to the method described by Voller et al., (1976).

Serum samples were examined for FMD viral specific IgG antibodies using in-house developed ELISA assay.

# 14 III. Results

Table (1) IFN-gamma for Montanide ISA 201 group (Group B) detected at 3 rd day following vaccination. Mean IFN-gamma cons. was (200pg/ml), Optical density (O.D.) was 0.765 and SP% was (40%). The highest level was at 14 Th day, with a cons. of (500pg/ml) and O.D. of 1.743 and SP% (93%). It was at protective level (>38%) till 42 days post vaccination. Tables (3) IFN-gamma for Montanide ISA 61 group (Group C) detected at 3 rd day following vaccination. Mean IFNgamma cons. was (250pg/ml), Optical density (O.D.) was as 0.881 and SP% was (46%). The highest level was at 14 Th day, with a cons. of (800pg/ml) and O.D. of 1.847 and SP% (97.5%).

It was at protective level (>38%) till 56 days post vaccination. Tables (4) IFN-gamma level of was undetectable in plasma of control unvaccinated group (Group D).

## 15 b) Evaluation of humeral immune response in calves vaccinated with FMD vaccines using SNT against FMDV serotypes (O,A&SAT2)

The humeral immune response of calves vaccinated with trivalent FMD vaccines(formulated with Montanide oil ISA 206,201 and 61) using SNT for FMD virus showed that protective neutralizing serum antibody titer for Montanide ISA 206 started at the 2 nd week post vaccination with average antibody titer of (1.5-1.6 &1.5 log 10 ) for (O, A & SAT2 ) respectively. The obtained antibody titer reached to the peak level at 10 th week post vaccination with average titers of (2.4 -2.7 &2.6 log 10 ). The protective neutralizing serum antibody titer for Montanide ISA 201 started at the 1 st week post vaccination with average antibody titer of (1.5-1.6&1.5 log 10 ) for (O, A&SAT2) respectively. The obtained antibody titer reached to the peak level at 10 th week post vaccination with average titers of (3.05-3.1 &3.05 log 10 ).The protective neutralizing serum antibody titer for Montanide ISA 61started at the 1 st week post vaccination with average antibody titer of (1.7, 1.8 &1.7 log 10 ) for (O, A&SAT2) respectively. The obtained antibody titer reached to the peak level at 10 th week post vaccination with average titers of (3.1-3.4&3.1 log 10 ). Tables (5) Table (5)

* = Antibody titers expressed as log 10 serum neutralizing antibody titer.

Protective level **??**1.5)

## 16 c) Evaluation of humeral immune response in calves vaccinated with FMD vaccines using ELISA against FMDV. serotypes (O,A&SAT2)

The protective antibody titer for FMD vaccine formulated with Montanide ISA 206 started at the 2 st week post vaccination with average antibody titer of (1.40 -1.50& 1.50 log 10 ) for O,A & SAT2 respectively . The obtained antibody titer reached to the peak level at 10 th week post vaccination with average titers of (2.90 -2.92 & 2.92log 10 ) for (O, A&SAT2) respectively.

The protective antibody titer for Montanide ISA 201 started at the 1 st week post vaccination with average antibody titer of (1.93 -1.95 &1.93 log 10 ). The obtained antibody titer reached to the peak level at 10 th week post vaccination with average titers of (3.12-3.15 &3.13 log10). The protective neutralizing serum antibody titer for Montanide ISA 61 started at the 2 nd week post vaccination with average antibody titer of (1.97-1.99 & 1.96 log 10 ). The obtained antibody titer reached to the peak level at 10 th week post vaccination with average titers of (3.32 -3.34 &3.33 log10).Table (6).

Table (6) : Antibody titers of calves vaccinated with inactivated trivalent FMD vaccine using ELISA against FMDV serotype (O, A and SAT2).

## 17 IV. Discussion

The first use of an oil adjuvant inactivated FMD vaccine was stated by Cunliffe and Graves **??**1963). Such vaccine was found to induce higher immune levels and protection in vaccinated cattle than that induced by the conventional aluminum hydroxide vaccines. So it could be considered an important tool in the control programs of FMD Bahnemann and Mesquita (1987) and Iyer et al., (2000). An adjuvant may act in one or more of five ways, based on current knowledge; namely, immunemodulation, presentation, induction of CD8+cytotoxic Tlymphocyte (CTL) responses, targeting, and depot generation. Addition to that adjuvant plays an important role in production of different lympho-kines such as various interleukins and INF-gamma according to Barnett et al., (2004) and Ebeid et al., (2011). The innate immune response induced by a viral infection in the upper respiratory tract, the macrophages present in the respiratory tract produce interferons (IFNs) upon stimulation of pattern recognizing surface receptors, causing alterations in local vascular walls, and providing recruitment and activating stimuli to antigen presenting cells and phagocytes Wilkins and Gale (2010). IFNs are also known as viral IFNs and secreted by virus infected cells with the function of blocking spread of virus to uninfected cells and have an important role in the host response to **??**MDV Summerfield et al., (2009) and that the ability of the virus to induce an IFN response may be related to the pathogenicity of different isolates of FMDV Santos et al., **??**2006) and Stenfeldt et al., (2011). To better characterize the immune response to FMD vaccines and to search for early markers predictive of induction of immune memory; must analyze the kinetics and magnitude of the antibody and cell-mediated immune responses to FMD vaccinesand further characterization of the antigen-specific CD4 + T-cell response better to be attempt by measuring IFN-gamma production **??**arr et al., (2013).

So, this study was performed for evaluation of FMD virus-specific cell-mediated immunity in cattle vaccinated with FMD vaccine adjuvanted with different Montanide oils using interferon-gamma Assay, in order to determine to any extent FMD trivalent vaccine is able to elicit a sterile immunity. **??**014) who observed that vaccine formulated with ISA 61 showed the highest specific IFN-gamma responses among the different ISA oil formulations, which can be observed at 9 weeks post vaccination. The results also showed that great variation

was observed between the vaccinated animal groups in INF-gamma production level depending on the adjuvant. From previous results, the quantity of IFN-gamma produced was significantly the highest in group (C) compared to the other groups from day 3 till day 63 post-vaccination .Also, the quantity of IFN-? produced in the plasma samples from vaccinated animals was significantly higher than the quantity produced in the samples from the unvaccinated control animals. From tables (5and 6) the results revealed that SNT and ELISA titers for different oil FMD vaccines agreed with Dar et al., (2013) who showed that Montanide ISA-201 adjuvanted vaccine induced earlier and higher neutralizing antibody responses as compared to the two other oil adjuvants, also were supported by Parida et al., (2006) who recorded that IFN-production assay could be used to support the established serological assays to confirm infection in a previously vaccinated herd. Our results also go in hand with the results obtained were consistent with the statement of Hamblin et al., (1986) who explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measure all classes of antibodies even those produced against incomplete and non-infectious virus.

The obtained results were in agreement with Parida et al., (2006) and Barnett et al., (2004) who showed that in a vaccine IFN-gamma response could be a useful indicator of the ability of a FMD vaccine to elicit a so-called sterile immunity in which subclinical infection is prevented. This early IFN-gamma production probably comes from NK cells activated by macrophage derived cytokines as part of the innate immune response.

Our results also were supported by Wu et al., (2003) and Diaz-San et al., (2010) who suggested that there is a complex interplay between IFN-induced immunomodulatory in protection of animals against FMDV.

Finally, conclusion from the obtained results through the present study it could be concluded that, all of the prepared vaccines were capable of stimulating a systemic gamma interferon response. Montanide ISA-61 adjuvanted vaccine induced early response, high cellular and humeral immunity and produced higher IFNgamma as compared to the two other adjuvants, while no systemic IFN-gamma was detected in plasma samples from the unvaccinated cattle.


Figure 1:

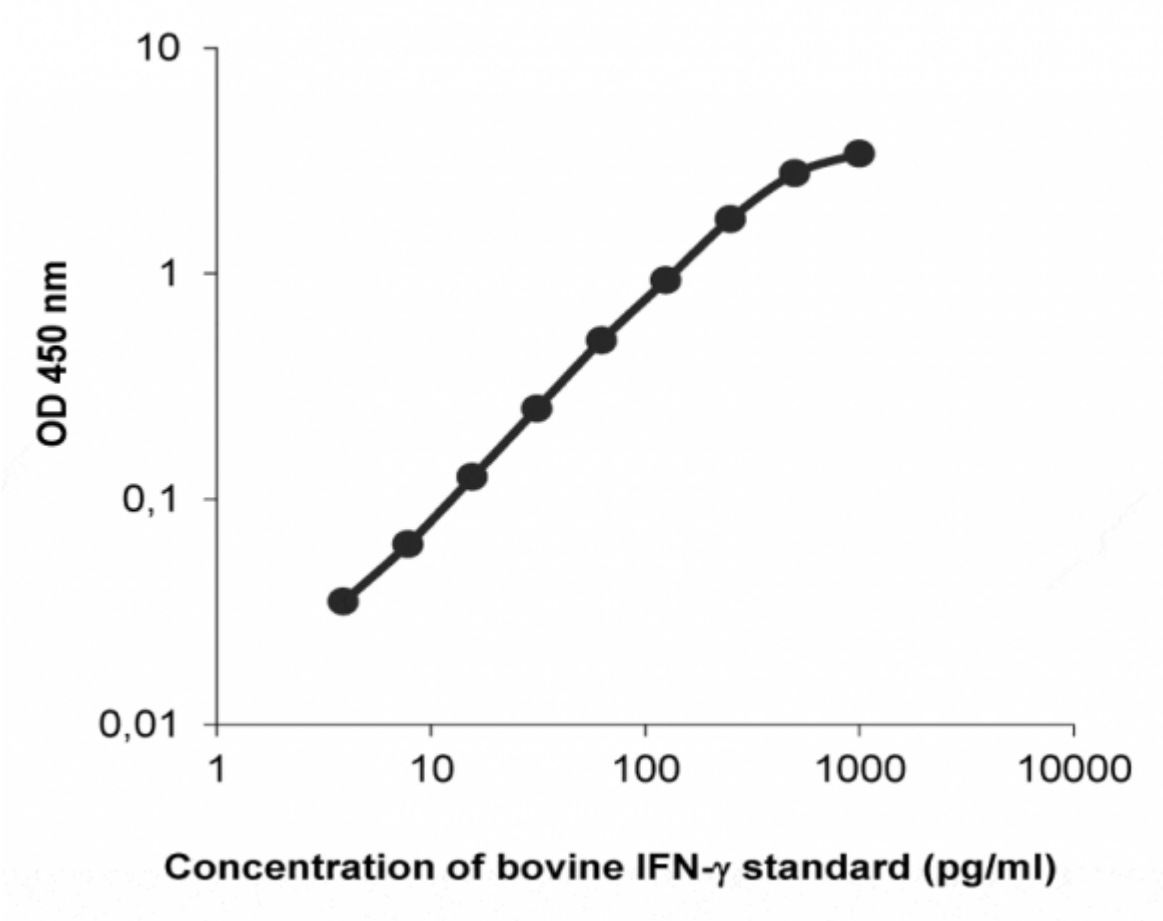


Figure 2:

| Standard | Bovine IFN-gamma Concentration (pg/ml) | O.D. at 492 nm | Mean O.D. at 4 nm |
|---|---|---|---|
| 1 | 1000.0 | 1.983 -1.977 | 1.980 |
| 2 | 500.0 | 1.701 -1.790 | 1.746 |
| 3 | 250.0 | 0.881 -0.876 | 0.879 |
| 4 | 125.0 | 0.462 -0.485 | 0.479 |
| 5 | 62.5 | 0.252 -0.258 | 0.255 |
| 6 | 31.3 | 0.144 -0.149 | 0.147 |
| 7 | 15.6 | 0.093 -0.096 | 0.095 |
| 8 | 7.8 | 0.067 -0.067 | 0.067 |
| Blank | 0 | 0.031 -0.028 | 0.030 |

*[Note: Chart(1) : Standard curve for typical data using Bovine IFN-gamma ELISA Kits]*

Figure 3: :

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| Days | IFN-gamma | Vaccinated cattle A1 | A2 | A3 | Mean | Mean Control group |
|---|---|---|---|---|---|---|
| | *cons. | 0 | 0 | 0 | 0 | 0 |
| 0 | O.D | 0.031 | 0.030 | 0.029 | 0.030 | 0.030 |
| | **SP% | 0% | 0% | 0% | 0% | 0% |
| 3 | ?cons. | 125.0 | 125.0 | 62.5 | 100 | 0 |
| | O.D | 0.462 | 0.462 | 0.258 | 0.394 | 0.030 |
| | SP% | 23% | 23% | 12% | 19.5% | 0% |
| 7 | ?cons. | 200.0 | 200 | 200 | 200 | 0 |
| | O.D | 0.761 | 0.765 | 0.763 | 0.763 | 0.030 |
| | SP% | 40% | 40% | 40% | 40% | 0% |
| 10 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 14 | ?cons. | 450 | 450 | 450 | 450 | 0 |
| | O.D | 1.572 | 1.572 | 1.572 | 1.572 | 0.030 |
| | SP% | 84% | 84% | 84% | 84% | 0% |
| 21 | ?cons. | 450 | 450 | 450 | 450 | 0 |
| | O.D | 1.572 | 1.572 | 1.572 | 1.572 | 0.030 |
| | SP% | 84% | 84% | 84% | 84% | 0% |
| 28 | ?cons. | 400 | 400 | 400 | 400 | 0 |
| | O.D | 1.402 | 1.400 | 1.401 | 1.400 | 0.030 |
| | SP% | 74% | 74% | 74% | 74% | 0% |
| 35 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 42 | ?cons. | 125.0 | 125.0 | 62.5 | 100 | 0 |
| | O.D | 0.462 | 0.462 | 0.258 | 0.394 | 0.030 |
| | SP% | 23% | 23% | 12% | 20% | 0% |
| 49 | ?cons. | 100 | 100 | 100 | 100 | 0 |
| | O.D | 0.396 | 0.392 | 0.394 | 0.394 | 0.030 |
| | SP% | 19.5% | 19.5% | 19.5% | 19.5% | 0% |
| 56 | ?cons. | 62.5 | 62.5 | 62.5 | 62.5 | 0 |
| | O.D | 0.258 | 0.254 | 0.256 | 0.256 | 0.030 |
| | SP% | 12% | 12% | 12% | 12% | 0% |

Figure 4: Table ( 2

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| Days * | IFN-gamma | Vaccinated cattle | | | | Mean Control group |
|---|---|---|---|---|---|---|
| | | A1 | A2 | A3 | Mean | |
| | *cons. | 0 | 0 | 0 | 0 | 0 |
| 0 | O.D | 0.031 | 0.030 | 0.029 | 0.030 | 0.030 |
| | **SP% | 0% | 0% | 0% | 0% | 0% |
| 3 | ?cons. | 200.0 | 200 | 200 | 200 | 0 |
| | O.D | 0.764 | 0.765 | 0.766 | 0.765 | 0.030 |
| | SP% | 40% | 40% | 40% | 40% | 0% |
| 7 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 10 | ?cons. | 500.0 | 500 | 500 | 500 | 0 |
| | O.D | 1.745 | 1.742 | 1.742 | 1.743 | 0.030 |
| | SP% | 93% | 93% | 93% | 93% | 0% |
| 14 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 21 | ?cons. | 500.0 | 450 | 500.0 | 650 | 0 |
| | O.D | 1.742 | 1.572 | 1.742 | 1.685 | 0.030 |
| | SP% | 92% | 83% | 92% | 89% | 0% |
| 28 | ?cons. | 400 | 400 | 400 | 400 | 0 |
| | O.D | 1.402 | 1.402 | 1.402 | 1.402 | 0.030 |
| | SP% | 74% | 74% | 74% | 74% | 0% |
| 35 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 42 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 49 | ?cons. | 125.0 | 125.0 | 125.0 | 125.0 | 0 |
| | O.D | 0.460 | 0.462 | 0.464 | 0.462 | 0.030 |
| | SP% | 23% | 23% | 23% | 23% | 0% |
| 56 | ?cons. | 100 | 100 | 100 | 100 | 0 |
| | O.D | 0.392 | 0.394 | 0.396 | 0.394 | 0.030 |
| | SP% | 19.5% | 19.5% | 19.5% | 19.5% | 0% |

*IFN-gamma cons. (pg/ml)
O.D.
at492 nm
** Sample-to positive %
SP% protection cutoff > 38%

Figure 5: Table ( 3

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| Days * | IFN-gamma | Vaccinated cattle | | | | Mean Control |
|---|---|---|---|---|---|---|
| | | A1 | A2 | A3 | Mean | group |
| | *cons. | 0 | 0 | 0 | 0 | 0 |
| 0 | O.D | 0.030 | 0.030 | 0.030 | 0.030 | 0.030 |
| | **SP% | 0% | 0% | 0% | 0% | 0% |
| 3 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 7 | ?cons. | 500.0 | 500 | 500 | 500 | 0 |
| | O.D | 1.745 | 1.745 | 1.745 | 1.745 | 0.030 |
| | SP% | 93% | 93% | 93% | 93% | 0% |
| 10 | ?cons. | 1000 | 650 | 800 | 800 | 0 |
| | O.D | 1.981 | 1.673 | 1.887 | 1.847 | 0.030 |
| | SP% | 105% | 88% | 100% | 97.5% | 0% |
| 14 | ?cons. | 800 | 800 | 500.0 | 800 | 0 |
| | O.D | 1.887 | 1.887 | 1.750 | 1.841 | 0.030 |
| | SP% | 100% | 100% | 92% | 97.5% | 0% |
| 21 | ?cons. | 500.0 | 450 | 500.0 | 650 | 0 |
| | O.D | 1.742 | 1.572 | 1.742 | 1.685 | 0.030 |
| | SP% | 92% | 83% | 92% | 89% | 0% |
| 28 | ?cons. | 500.0 | 450 | 500.0 | 650 | 0 |
| | O.D | 1.742 | 1.572 | 1.742 | 1.685 | 0.030 |
| | SP% | 92% | 83% | 92% | 89% | 0% |
| 35 | ?cons. | 400 | 400 | 400 | 400 | 0 |
| | O.D | 1.402 | 1.400 | 1.401 | 1.400 | 0.030 |
| | SP% | 74% | 74% | 74% | 74% | 0% |
| 42 | ?cons. | 400 | 400 | 400 | 400 | 0 |
| | O.D | 1.402 | 1.400 | 1.401 | 1.400 | 0.030 |
| | SP% | 74% | 74% | 74% | 74% | 0% |
| 49 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |
| 56 | ?cons. | 250.0 | 250.0 | 250.0 | 250.0 | 0 |
| | O.D | 0.881 | 0.881 | 0.881 | 0.881 | 0.030 |
| | SP% | 46% | 46% | 46% | 46% | 0% |

*IFN-gamma cons. (pg/ml)
O.D.
at492 nm
** Sample-to positive %SP% protection cutoff > 38%

Figure 6: Table ( 4

| | SNT titers of vaccinated animal groups | | | | | | | | | Control group |
|---|---|---|---|---|---|---|---|---|---|---|
| Time post vaccination | | Group A (ISA 206) | | | Group B (ISA 201) | | | Group C (ISA 61) | | |
| | O | A | SAT2 | O | A | SAT2 | O | A | SAT2 | |
| 0 | 0.15* | 0.12 | 0.12 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| 1 week | 0.9 | 1.2 | 0.9 | 1.5 | 1.6 | 1.5 | 1.7 | 1.8 | 1.7 | 0.3 |
| 2 week | 1.5 | 1.6 | 1.5 | 1.7 | 1.8 | 1.7 | 1.8 | 2.1 | 1.8 | 0.3 |
| 3 week | 1.8 | 1.8 | 1.8 | 2.1 | 2.4 | 2.1 | 2.4 | 2.4 | 2.4 | 0.3 |
| 4 week | 2.1 | 2.1 | 2.1 | 2.4 | 2.4 | 2.4 | 2.7 | 2.7 | 2.7 | 0.6 |
| 6 week | 2.1 | 2.4 | 2.1 | 2.4 | 2.7 | 2.4 | 2.7 | 2.9 | 2.7 | 0.9 |
| 8 week | 2.4 | 2.4 | 2.4 | 2.7 | 2.9 | 2.7 | 3.05 | 3.1 | 3.05 | 0.9 |
| 10 week | 2.4 | 2.7 | 2.6 | 3.05 | 3.1 | 3.05 | 3.1 | 3.4 | 3.1 | 0.9 |
| 12 week | 2.4 | 2.4 | 2.4 | 2.7 | 2.9 | 2.7 | 3.05 | 3.1 | 3.05 | 0.6 |
| 14 week | 2.1 | 2.1 | 2.1 | 2.7 | 2.7 | 2.7 | 2.8 | 2.8 | 2.8 | 0.6 |
| 16 week | 2.1 | 2.1 | 2.1 | 2.4 | 2.7 | 2.4 | 2.7 | 2.8 | 2.7 | 0.6 |
| 18 week | 2.1 | 2.1 | 2.1 | 2.4 | 2.4 | 2.4 | 2.6 | 2.7 | 2.6 | 0.6 |
| 20 week | 1.8 | | | | | | | | | |

Figure 7: :

(

1) and Chart (1) show the typical data using Bovine IFN-gamma ELISA Kits for 8 slandered solutions beside the blank one. Bovine IFN-gamma Concentration (pg/ml) with the respectively O.D. at 492 nm.

From Tables (2, 3 and 4) no systemic IFN-gamma was detected in plasma samples from the unvaccinated cattle. IFN-gamma for Montanise ISA 206 group (Group A) detected at 7 th day following vaccination, that results agreed with Stenfeldt et al., (2011) who observed that within seven days of vaccination with FMD oil vaccine, IFN-gamma production was observed and supported with Cavalcanti et al., (2012) and Bucafusco et al., (2015) they found that on day 7 both CD4 + and CD8 + T cell populations produced IFN-gamma .The obtained results also in agreement in some points with Habjanec et al., (2008) who stated that ISA206 formulations were less effective in inducing INF-gamma. IFN-gamma for Montanise ISA 201 group (Group B) detected at 3 rd day following vaccination and that results agreed with Dar et al., (2013) who observed that Montanide ISA-201 adjuvanted vaccine induced earlier and higher immune response in vaccinated animals, and supported with Gurung et al., (2014) who reported that vaccine formulation with the antigen and Montanide? ISA 201 adjuvant produced strong specific IFN-gamma responses in a high proportion of the vaccinated animals. IFN-gamma for Montanide ISA 201 group (Group B) detected at 3 rd day following vaccination and that results agreed with Dar et al., (2013) who observed that Montanide ISA-201 adjuvanted vaccine induced earlier and higher immune response in vaccinated animals, and supported with Gurung et al., (2014) who reported that vaccine formulation with the antigen and Montanide? ISA 201VG adjuvant produced strong specific IFN-gamma responses in a high proportion of the vaccinated animals. The results also come parallel and in agreement with what obtained by Dong et al., (2013) who reported that the efficacy of the FMD vaccine emulsified with ISA 201 was better than which with ISA 206. IFN-gamma for Montanide ISA 61 group (Group C) detected at 3 rd day following vaccination and that results agreed with Gurung et al., (

Figure 8: Table (

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