Technical Booklet

Information from Phibro Technical Services

MB-1™ IBD

Live Gumboro Vaccine

An Evolution in IBD Hatchery Vaccination

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Introduction

Infectious bursal disease (IBD) is one of the most important diseases of poultry occurring worldwide. Until the late 1980s, infectious bursal disease was controlled by priming the parent birds with a live attenuated vaccine, followed by an inactivated vaccines. This vaccination regime induced a high level of antibodies in vaccinated breeders that delivered maternal antibodies that protected progeny chicks against field challenges. However, with the emergence of variant and very virulent strains of infectious bursal disease virus (vvIBDV) it became necessary to also actively vaccinate young chicks with live attenuated IBD vaccines at the farm. Veterinarians and growers quickly realized that the presence of maternally derived antibodies (MDA) at various levels, influence the application timing and vaccine efficacy. Hence, right timing of vaccination is critical, and must be done when MDA level drops below sufficient level (breakthrough titer) to allow replication of vaccine virus in bursa and to develop the desired immunity.

In commercial settings of Indian poultry industry, routine monitoring of MDA level and determination of the right age of vaccination in all classes viz, broiler, layer & breeder is cumbersome!

With the development of various forms of hatchery adapted IBD live vaccine - where day old chicks could be vaccinated at convenience in hatchery is a definitive solution. The user has complete control on vaccination process - right dosing and accuracy of 100% chicks to be vaccinated.

So far, there were two types of commercial IBD hatchery vaccines:

1. Immune complex vaccines
2. Vectored vaccines

Limitations of current types of hatchery IBD vaccines

1. Both, immune complex and vectored vaccines has primary disadvantage of delayed onset of immunity. (Ashash et al.)
2. Vectored vaccine is not a live Gumboro vaccine virus but an HVT virus presenting the VP2 protein of the Gumboro virus and has following more limitations:
   - Expresses only VP2 protein and therefore, immune responses against the vaccine misses out on other important epitopes like VP3 or other epitopes in whole capsid.
   - Neutralising epitopes in VP2 is conformational dependent and hence, denatured VP2 does not induce protection in chickens (Fahey et al.,1989)
   - Denatured and renatured VP2 also lost the ability to induce neutralising antibodies in chickens (Oppling et al., 1989)
3. Immune complex IBD vaccine is a suspension of a live attenuated Gumboro virus mixed with antiserum against IBD in well-defined proportions. The vaccine viruses are covered and consequently protected from recognition by the immune system of the chickens by specific immunoglobulins (MDA). Efficacy of Immune complex vaccine is formulation dependent i.e. proportion of antiserum & vaccine virus in formulation of a product determines it's uptake by immune system and thereby onset of immune response in chicks with different MDA levels.

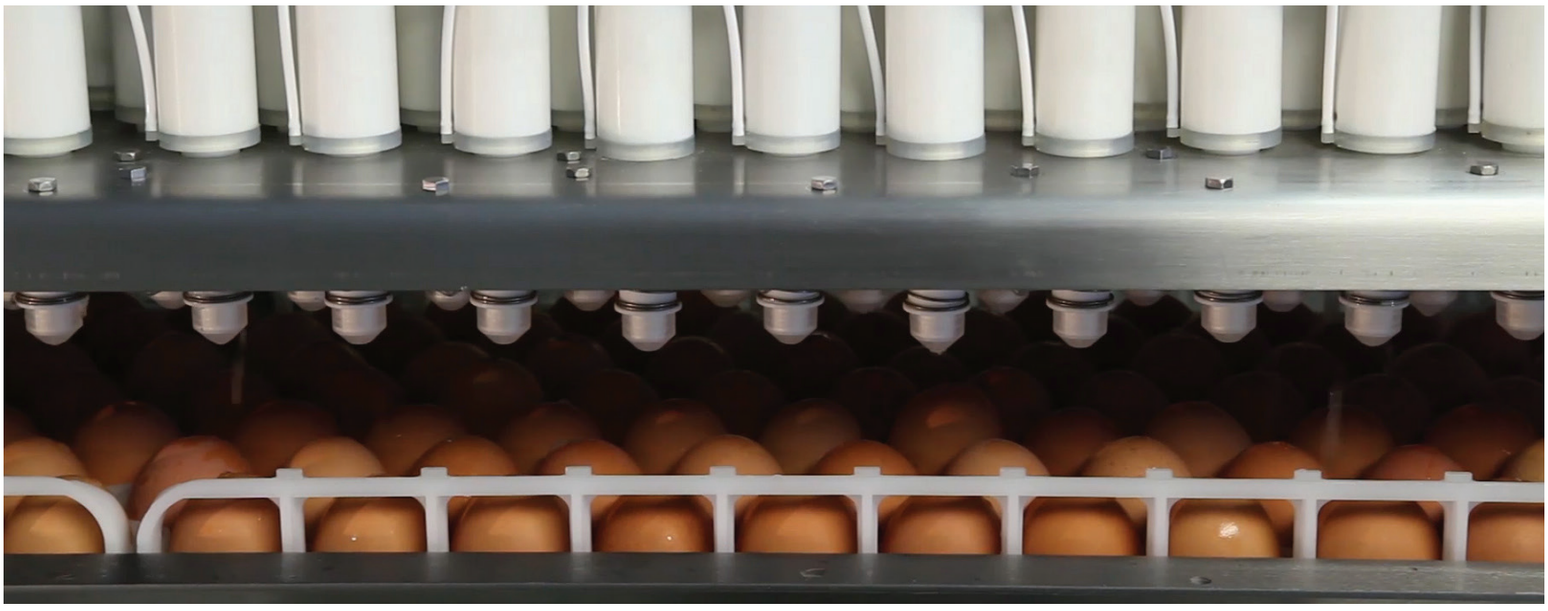
Reference

1. Fahey, K.J., Erny, K. & Crooks, J. (1989). A conformational immuno- gen on VP2 of infectious bursal disease virus that induces virus- neutralizing antibodies that passively protect chickens. Journal of General Virology, 70, 1473-1481.
2. O¨ ppling, V., Mu¨ ller, H. & Becht, H. (1989). Heterogeneity of the antigenic site responsible for the induction of neutralizing antibodies in infectious bursal disease virus. Archives of Virology, 119, 211-223.
3. Ashsah U et al (2013) Evaluation of different vaccination technologies against very virulent IBDV - dose one vaccine solution fits all the different IBDV field strains and different husbandries?" Proceedings of WVPA Nantes, France.

MB-1 is a novel, infectious bursal disease live attenuated virus vaccine, originated from the M.B. strain, adapted for in-ovo or subcutaneous (SC) injection at the hatchery.

MB-1 advantages

- MB-1 is a naked live virus unlike Vectored vaccines carrying the VP2 subunit or the immune complex (Icx) vaccines where the virus is coated with anti - Gumboro antibodies.
- MB-1 onset of immunity compared to Icx and vectored/recombinant vaccines is earlier. (Chart 1, table 1 and table 2)
- MB-1 attenuation level enables the vaccine to successfully protect against any form of IBD including vvIBDV and at the same time is extremely safe.
- MB-1 can be applied in-ovo at 18.5 days of incubation or by subcutaneous injection in day old chicks.
- MB-1 does not generate immunosuppression.
- MB-1 adjusts to varying (high to low) IBD maternally derived antibodies of chicks in a way that individual chicks are ensured of having vaccinated at the right age thus immunity after MB-1 is with a small window of susceptibility.



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Data from Phibro Technical bulletin; Brazil 2017 trial

Table 1. IBD ELISA titers

Age(d)	MB-1™	Immune-complex vaccine
1	5045.00	5634.00
7	1940.00	2231.00
14	481.53	691.25
17	131.80	193.44
21	89.53	71.81
24	50.07	65.36
28	**898.13[a]**	**111.50[b]**
32	**2752.73[a]**	**650.06[b]**
36	2667.53	2358.25
40	2910.88	3154.69

Student t-test, two tailed assuming unequal variance, P≦0.05
different small letters indicate statistical significance

Chart 1. Elisa titers

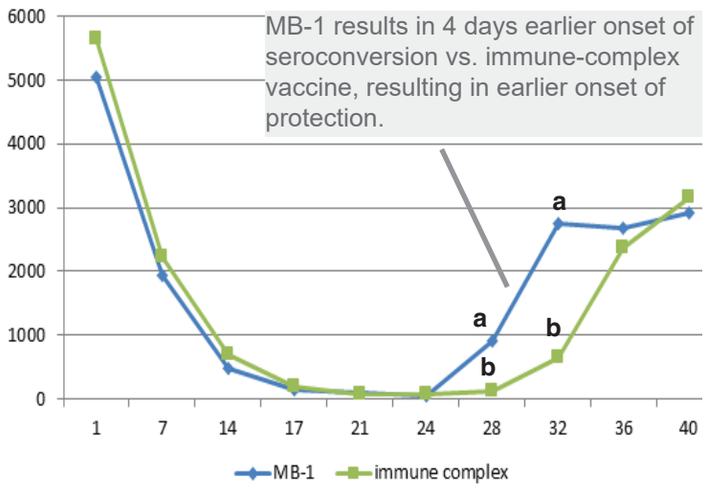


Table 2. Results of PCR from bursal samples

Percent of positive for vaccine strain

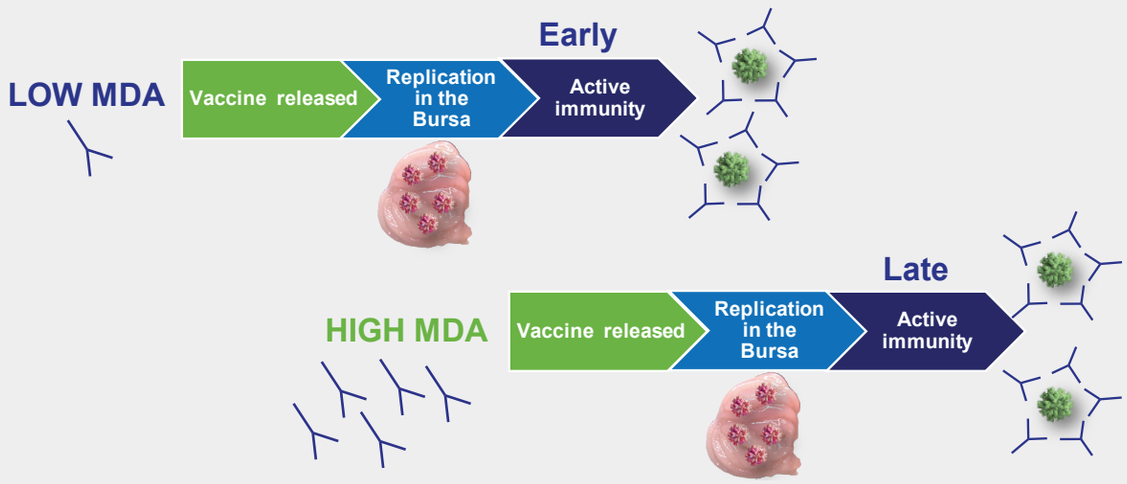
Age (d)	MB-1™ (%)	Immune complex (%)
24	33.33	0
28	100.00	33.33
32	100.00	100.00
36	100.00	100.00
40	100.00	83.33

At 24 days of age the M.B. strain was identified in 33.33% of the samples followed by positive identifications in 100% of the samples up to 40 days of age.
The immune-complex vaccine strain was identified 4 days later, at 28 days of age in 33% and onwards up to 100% of the tested samples.

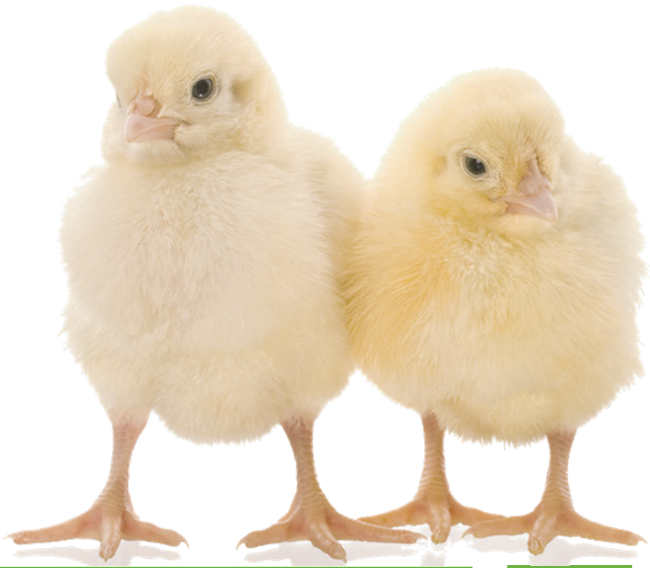
MB-1 Mode of action

After injection, the MB-1 vaccine viruses are coated with the chick's maternal derived antibodies (MDAs). The virus is engineered in such a way that instead of neutralizing the vaccine virus, it prevents the same from replicating too early in the young chick bursa. The naturally coated virus in vivo by chick MDA are stored and released post decay of MDA to appropriate level. Once the virus is released, it can replicate in the bursa like any other regular live vaccine. This action is happening in every chicks at a different time according to each chick's individual MDA level.

Chart 2. Mode of action



For additional information please refer to Phibro technical bulletin; Mode of action



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Field Trial in India, 2019-20

Objectives:

1. *Potency:* To compare the IBD ELISA titers in broilers vaccinated with MB-1 to that of broilers vaccinated with 228E in field/farm and broilers vaccinated with immune complex at the hatchery.
2. *Safety:* To compare the impact on the bursa of Fabricius in broilers vaccinated with MB-1 to that of broilers vaccinated with 228E and broilers vaccinated with immune complex at the hatchery by Bursa to Body Weight Ratio.
3. *Efficacy:*
   - To compare the efficacy against IBD in broilers vaccinated with MB-1 to that of broilers vaccinated with 228E and broilers vaccinated with immune complex at the hatchery by PCR.
   - To compare the performance results and production cost analysis of broilers vaccinated with MB-1 to that of broilers vaccinated with 228E and broilers vaccinated with immune complex at the hatchery.

Material & Methods

Vaccines

Type of vaccine	Vaccine name
IBD	MB-1
	D 228E (Conventional live)
	Immune Complex (based on 2512)
ND	Inactivated ND vaccine
	VH clone
	Lasota Clone

Injector: Desvac Dovac®
Chicks: Cobb 430Y straight run flock
Housing type: Open sided
Management: Feeding, drinking water, ventilation and farming management were deployed as per standards of integration of commercial broilers.

4. Trial Design

Table 3. Trial Design

Farm house	No. of Chicks	Vaccines	Vaccination age (days)	Administration route
Farm 1 House 1	4600	MB-1	1	0.1ml SC
		ND inactivated	1	0.1ml SC
		ND live	5	DW
		ND live	18	DW
Farm 1 House 2	4600	Immune complex	1	SC
		ND inactivated	1	0.1ml SC
		ND live	5	DW
		ND live	18	DW
Farm 2 House 1	2500	MB-1	1	0.1 ml SC
		ND inactivated	1	0.1ml SC
		ND live	5	DW
		ND live	18	DW
		ND live	28-29	DW
Fram 2 House 2	2500	ND inactivated	1	0.1ml SC
		ND live	5	DW
		228E	14	DW
		ND live	18	DW
		ND live	28-29	DW

5. Sampling & Analysis

- At 4d of age Blood samples from 20 commercial DOC were taken for the evaluation of IBD maternal antibodies (MAb) by IDEXX XR ELISA kit.
- At Days, 14, 21, 28 & 40, 20 blood samples were taken from each house for the evaluation of IBD by IDEXX XR ELISA kit.
- At Days 17, 21, 24, 28, 32 & 40, 6 birds from each house were euthanized for bursa body weight ratio & PCR.
- IBD ELISA titer, Bursa body weight ratio (B2BWR), PCR, performance parameters - PCR, EEF, DWG and mortality were measured for safety & efficacy assessment of vaccines

6. Statistical analysis

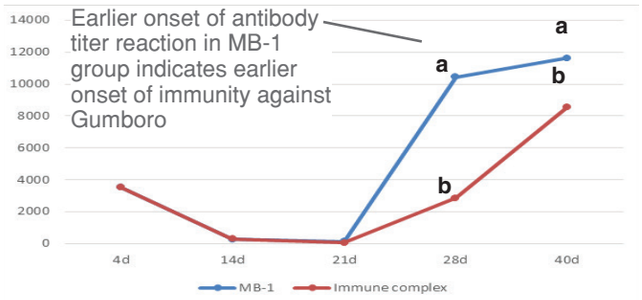
Data were analysed by Student t-test, two tailed assuming unequal variance, P≦0.05

Table 4. IBD ELISA titers: Farm 1

Farm 1		
Age	**MB-1 House 1**	***IC House 2**
4d	3505	3505
14d	269	287
21d	120	58
28d	10438[a]	2839[b]
40d	11622[a]	8572[b]

*IC- Immune complex
Small letters indicate statistical significance

Chart 2. Average IBD Elisa titers



Small letters indicates siginficant statistical differences

Table 5. Farm 1 Bursa to Body Weight Ratio

Farm 1		
Age	**MB-1 House 1**	***IC House 2**
17d	0.26	0.23
21d	0.22	0.24
24d	0.23	0.26
28d	0.08	0.06
32d	0.05	0.06
40d	0.05	0.05

*IC- Immune complex

Chart 3. Average Bursa to Body Weight Ratio

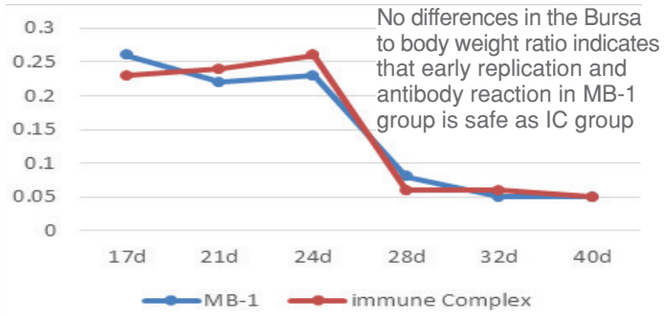


Table 6. Results of PCR from Bursal smears on FTA card

Farm 1		
Age	**MB-1 House 1**	***IC House 2**
17d	Negative	Negative
21d	M.B.	2512
24d	M.B.	2512
29-8d	M.B.	2512
32d	M.B.	2512
40d	M.B. Weak	2512

*IC- Immune complex

Both vaccines viruses MB-1 and 2512 appeared in Bursa smears on 21 days of age, however MB-1 group had a faster and higher antibody reaction. Therefore, we can conclude that MB-1 started to replicate earlier than 21 days of the bursa of the birds

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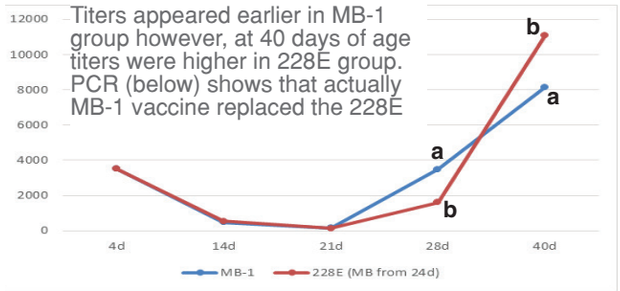
Farm 2 Results

Table 7. IBD ELISA titers: Farm 2

Farm 2		
Age	**MB-1 House 1**	**228E House 2**
4d	3505	3505
14d	474	548
21d	151	128
29d	3496[a]	1613[b]
40d	8160[a]	11100[b]

small letters indicate statistical significance

Chart 4. Average IBD Elisa titers



Small letters indicates siginficant statistical differences

Table 8. Farm 2 Bursa to Body Weight Ratio

Farm 2		
Age	**MB-1 House 1**	**228E House 2**
17d	0.23	0.28
21d	0.15	0.17
24d	0.15	0.2
28d	0.12	0.15
32d	0.04	0.05
40d	0.04	0.05

Chart 5. Average Bursa to Body Weight Ratio

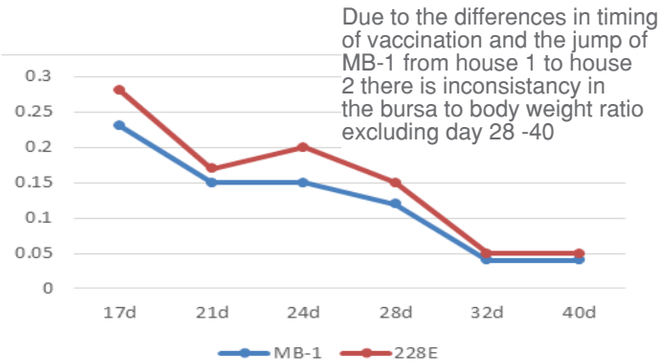
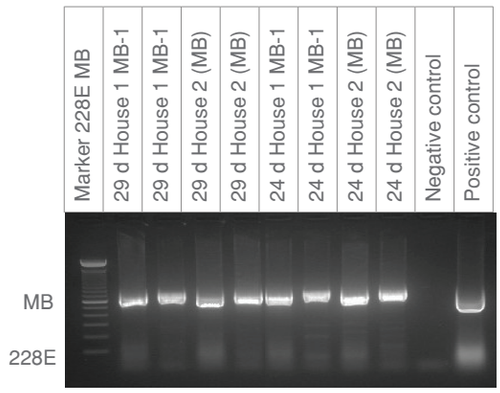


Table 9. Results of PCR from Bursal smears on FTA card

Farm 2		
Age	**MB-1 House 1**	**228E House 2**
17d	Negative	228E
21d	M.B.	Negative
24d	M.B.	M.B.
29-8d	M.B.	M.B.
32d	M.B.	M.B.
40d	M.B. Weak	M.B.

PCR results at 24 and 29 days of age showing only MB-1 strain in both houses.



228E vaccine was applied at 14 days of age and was found in the bursa at 17 days of age, but no at 21 days of age onwards. Instead we see MB-1 strain from 24 day onwards.

This data indicates that 228E was unable to protect the birds from cross contamination with MB-1 vaccine.

We can conclude that MB-1is with an excellent spreading ability adding to it's other advantages.

Table 10. Farm 1 Broilers performance results at 34d

Farm 1			
No	Parameter	MB-1 House 1	IC House 2
1	Breed	Cobb 430 Y	
2	Chicks at 34d	4590	4588
3	Mean Age	34	34
4	Feed consumption	2.903	2.954
5	Feed Intake %	96.35	98.04
6	Body weight	2.03	1.97
7	Day Gain	59.71	57.94
8	FCR	1.430	1.500
9	CFCR	1.424	1.506
10	Mortality	2.84	3.07
11	Chick cost	12.95	13.22
12	Feed cost (per kg of bird)	51.17	52.92
13	Medicine cost	0.5	0.51
14	Admin cost	6.22	6.35
15	Overall Production cost	70.84	73.00
16	EEF	402.28	378.71

Table 11. Farm 2 Broilers performance results at 34d

Farm 2			
No	Parameter	MB-1 House 1	228E House 2
1	Breed	Cobb 430 Y	
2	Chicks at 35d	2492	2463
3	Mean Age	35	35
4	Feed consumption	3.008	3.014
5	Feed Intake %	94.06	94.25
6	Body weight	2.1	2.04
7	Day Gain	60	58.29
8	FCR	1.430	1.480
9	CFCR	1.410	1.472
10	Mortality	3.97	4.99
11	Chick cost	12.43	12.93
12	Feed cost (per kg of bird)	53.01	54.68
13	Medicine cost	0.48	0.52
14	Admin cost	5.96	6.21
15	Production cost	71.87	74.34
16	EEF	394.65	371.16

** Chick cost = (No. of chicks X Actual chick cost)/ Total meat produced in Kg.*

Performance results of both MB-1 groups are significantly better than control groups

Conclusion

1. MB-1 vaccinated birds showed early and higher immune response (antibody titer) than conventional live vaccine (D228E) and immune complex vaccine (Ceva). The early immune response stems from the fact that the vaccine virus (MB-1) appeared early in bursa corroborated by PCR.
2. MB-1 vaccine was extremely safe as it had registered similar bursa-body weight ratio than immune complex vaccine.
3. MB-1 vaccine had improved growth performance parameters like FCR & EEF better than immune complex vaccine and 228E.

Global Field Trials

Trials took place in different countries, seasons and management conditions from deep-litter to fully controlled houses
MB-1 was injected In-OVO or SC
All trials included monitoring of immune parameters, PCR and performance
All results are statistically analyzed.
Not even a single failure to provide protection was noticed.

Country	Year/ No of trials	Birds
Israel	2016/ 1	12,000
RSA	2016-2018 / 8	2,714,000
Argentina	2017-2019 / 17	12,279,000
Brazil	2017-2018 / 5	1,667,491
Russia	2019 / 2	514,560
Indonesia	2019 / 1	38,000
India	2019 / 2	16,150
TOT	2016-2019 / 36	17,229,201

Application	Number
SC	4,916,060
In-OVO	12,293,141
TOT	17,209,201

Compared to	Number
Immune complex ICX	4,471,000
HVT-IBD	512,000
Conventional Live	1,840,060
HVT-IBD + Live	392,000
Mixed ICX /HVT-IBD	2,900,000
No comparison	207,491
TOT	10,452,191

Field trial Israel 2016

MB-1 vs. Live vaccine (17d) onset of immunity parameters

Bursa to body weight ratio

Age (d)	MB-1	Live
21	2.27	1.65
24	1.62	2.18
28	1.63	1.14
35	0.77	0.41

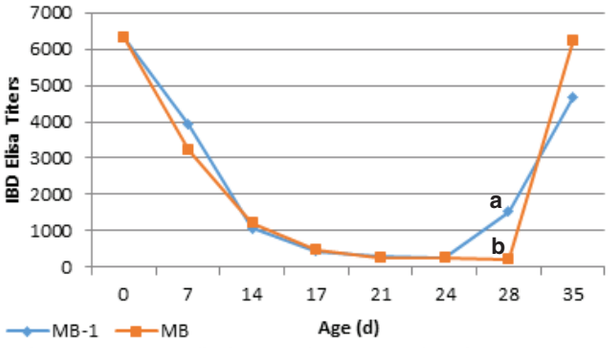
The MB-1 vaccine started the replication at about 14-15 days of age, 3 days earlier than the application of the live vaccine of age.

PCR results

Age (d)	MB-1 % pos.	Live % pos.
21	33%	17%
24	33%	0%
28	83%	100%
35	100%	100%

PCR results indicate at 24, 28 & 32 days of age, the vaccine strain was found in the MB-1 group and at 28 & 35 days in the live vaccine group. The differences of positive vs. strain are due to lack of material for sequencing.

Chart 6. IBD ELISA titers



Student t-test, two tailed assuming unequal variance, P≦0.05
different small letters indicate statistical significance

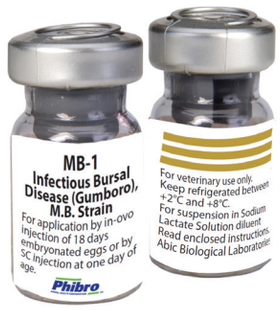
At day 28 the titers in the MB-1 group were significantly higher than the live vaccine group.
It is clear that MB-1 replicated in the bursa before the live vaccine.

Field trial Brazil 2017

Performance results

MB-1 group performance results are as good as or better than IC immune complex performance results

Group	MB-1	IC
Slaughtered	35,384	35,275
Birds/m^2	13.6	13.6
Kg/m	40.69	39.40
Age	42.67	41.89
Index	431.72	427.01
FCR	1.626	1.620
Mortality %	4.37	4.66
FCR – 2.7 Kg	1.519	1.535
BW	3.128	3.038
DWG	73.30	72.52



Main conclusions global field trials 2016 - 2019

1. Successful trials in Argentina, Brazil, RSA, Israel, Russia, Indonesia, India
2. Avg. Maternal immunity range in the trials: 10,000- 3000(IDEXX XR Elisa)
3. Maternal immunity range 800-12,000 (Elisa)
4. MB-1: 4 days earlier onset of immunity when compared to immune-complex vaccine (measured by PCR, serology & Histopathology)
5. MB-1: onset of immunity is the same as live conventional vaccine given at the correct timing
6. The immunity against other diseases is not compromised – NO immunosuppression observed with MB-1
7. MB-1 has the same Bursal effect as Immune-complex vaccine and live conventional vaccines
8. Good hatchability and performance after IN-OVO injection of MB-1 in large scale field trials
9. MB-1 is Compatible with other live vaccines given by spray, in OVO or SC injection
10. Good broiler performance parameters

MB-1 the ultimate Gumboro solution

Vaccine	Live	Immune complex	Vector	MB-1 best of all worlds
Main Advantage	Early protection	Hatchery application	Hatchery application	▪ Hatchery application ▪ Individual vaccination ▪ Early protection
Main Disadvantage	Application	Delayed OOI	Delayed OOI Not a live Gumboro	

Preparing the MB-1™ vaccine for injection

MB-1 is a live attenuated IBD Vaccine, developed for in-ovo (0.1 to 0.05 mL / dose) or one day old subcutaneous injection (0.2 mL / dose) in the hatchery.

MB-1 well-vaccinated chickens are protected against the most challenging Infectious Bursal Disease strains in the field.

Material Check list

- MB-1 Vaccine
- Diluent
- 5 mL sterile syringe with an 18 G needle
- 3 mL syringe with an 18 G needle
- Sterile gloves
- 70% Alcohol
- Sterile gauze
- Sterile injectable dye
- Marker and stickers

Preparation instructions

Step 1

Inspect the diluent

Do a visual inspection of the diluent to make sure there is no material floating inside of the diluent.

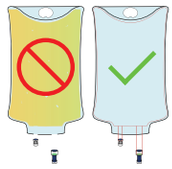
1. Match the MB-1 dose presentation to the diluent's presentation, aligned with the volume administered by the equipment and route to be used.
2. Follow the vaccination equipment directions.
3. Only dilute the amount of vaccine that will be administered right away.

If using a clear diluent:

Make sure the diluent remains clear in color.

If using a Marek's diluent:

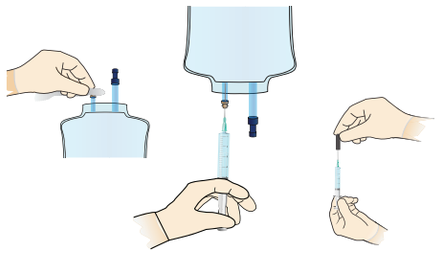
Do not use if it has turned turbid or changed to yellow color.



Step 2

Use dedicated area for vaccine preparationt

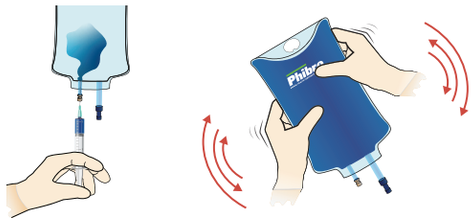
In a clean, closed, temperature-controlled area (25°C) specifically dedicated for the vaccine preparation, put on sterile gloves and disinfect the container's stopper with 70% Alcohol.
Take 2 mL of the diluent with the 5 mL syringe, place the cover back on the needle and set aside for future steps.



Step 3

Mix only approved additives (Skip step if no additives are used)

If any additives are to be used, such as a (blue) dye to verify the correct administration, mix them prior to the vaccine dilution.
With the 3 mL syringe take the volume recommended by the dye's manufacturer and inject it into the diluent's bag, shake the bag thoroughly and allow a couple of minutes for the pH to stabilize.



Warning: Never use expired vaccine, diluent, or additives (dye).

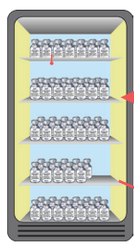
Step 4

Take only the MB-1 vaccine vials to be administered immediately out of the refrigerator

Keep at 2°C to 8°C

Note: Do Not Freeze

Check and log temperature twice a day



Use vaccine according to the expiration date. First in - First out.

Never leave the vaccine outside of the refrigerator

Step 5

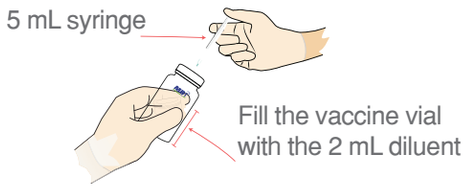
Remove aluminum cap and disinfect the rubber

Remove the aluminium cap and disinfect the rubber stopper with sterile gauze containing 70% alcohol. Let dry before next step.

Step 6

Fill the MB-1 vaccine vial with the 2 mL diluent

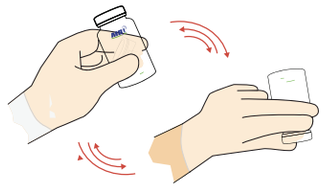
Take the 5 mL syringe prepared in the beginning of the process and fill the MB-1 vaccine vial with 2 mL diluent.



Step 7

Invert, dissolving the vaccine

Gently invert the vial making sure all of the vaccine cake has been dissolved. Once thoroughly mixed retrieve ALL of the newly mixed vaccine and diluent from the vial making sure nothing remains.



Step 8

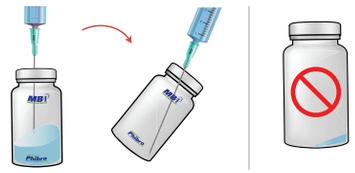
Inject the diluted vaccine into the diluent bag

Inject the newly mixed vaccine into the diluent bag.
Once injected, gently shake the bag.

Step 9

Retrieve 3 mL from the diluent bag and rinse the MB-1 vial

Inject the retrieved diluent into the MB-1 vial, rinse and draw ALL the liquid from the vial. At this stage the vial should look completely clean of any vaccine remains.



Make sure ALL the diluent with the vaccine is collected from the vial before disposing the vial. Dispose the empty vaccine vial as directed by local authorities for biologic materials.

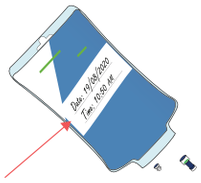
After rinsing the vial, inject the diluent back into the bag. Keep the needle inside the port and gently draw diluent back and forth 2-3 times to rinse the needle.

Remember: Small quantities of the diluent left in the vial is one of the most common errors during any vaccine preparation.

Step 10

Write the date and time the vaccine was prepared

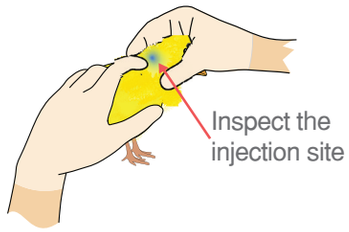
Apply a label and write the date and time the vaccine was prepared. The entire vaccine must be used within 60 minutes of preparation.



Step 11

Monitoring the injection quality

During vaccination, verify the injection quality by inspecting the injection site (Color under the skin) of 100 chicks or, if applying in-ovo, run an audit periodically at the site of the injection and look for the vaccine in the embryo's amnion, intramuscular or SC.

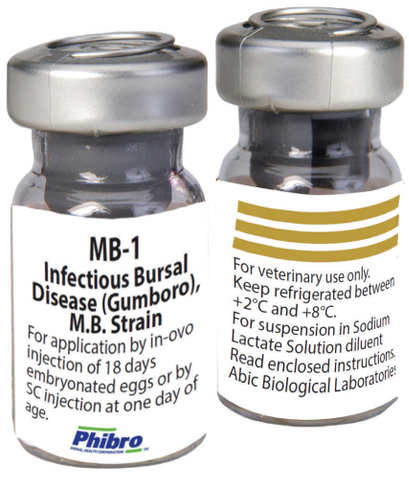


Important notes

The Phibro Technical Team can help organize vaccine administration assessments and share best practices for proper vaccination. Make sure to coordinate regular visits of Phibro's Tech Support Team. Keep records of the MB-1 serial administered to the different baby chick flocks, the time and date of the administration, the number of vaccinated birds, the name of the responsible person to prepare the vaccine, the record of any other additive mixed with the vaccine, the diluent's serial number, as well as any other relevant information.

MB-1 is a novel solution to gumboro disease that features convenience of hatchery application either S.C. or in ovo and being a native virus adjusts automatically for releasing in bursa at the level of individual chick. It's engineered to protect each & every chick in a flock with varying MDA levels at correct age & time.

The attenuation level of MB-1 ensures effective protection against all pathotypes of IBDV and at the same time being extremely safe.



TAbic® family – TAbic® V.H., TAbic® IBVAR206 and TAbic® IB Var, TAbic® H-120 and TAbic® M.B.

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