

NATIONAL DAIRY DEVELOPMENT BOARD

Disease Testing Protocols and Health Guidelines

For Progeny Testing (PT) and Pedigree Selection (PS) Projects



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1 Definitions

Bull Calf	A male cattle or buffalo which has not yet reached sexual maturity born out of nominated mating in the field.
Selected calf	Calves that conform to breed characteristics, are healthy without clinical symptom of disease, have no physical deformities and whose dam meets the 'elite dam'/ probable elite dam criteria (on the basis of initial records, preferably five monthly records).
Minimum Standards for Production of frozen semen (MSP) diseases	The set of diseases – the causative organism of which should not be present in the calf. These diseases include Bovine Brucellosis, Tuberculosis (TB), Paratuberculosis (JD) and Infectious Bovine Rhinotracheitis (IBR) - only when an IBR free herd is required.
Pre-Quarantine station	A farm where bull calves below 3 months of age are kept in isolation before shifting them to a quarantine station (while the results of their dams are awaited and, till the male calves are at least 3 months of age), to ensure that the calves and their dams are negative for all the MSP diseases. Occasionally, calves above 3 months are also maintained in isolation if their results or dam's results are pending but urgent procurement from field is required. The calves are shifted to the quarantine only if they and their dams are negative for all the MSP diseases (IBR may be an exception).
Rearing centre (For Pedigree Selection (PS) programmes only)	An establishment where bull calves of indigenous cattle and buffalo breeds usually aged 6 months and above are maintained after they and their dams have already been tested negative for all MSP diseases in the field. Here they are tested at least twice with negative results before they are moved to quarantine station, the last test being within 15 days of shifting to quarantine station.

Quarantine Station	A farm where bull calves above 3 months of age are kept in isolation, after they and their dams have already been tested negative for all MSP diseases either in field or while in pre-quarantine and where at least two rounds of testing are done for all MSP diseases with negative results, before they can be shifted to a rearing station.
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2 Testing protocols

Testing protocols are mandatory tests to be carried out on male calves selected through PS and PT programmes in order to minimize the chances of transmitting diseases through frozen semen.

Test details

The details on the tests to be carried out is summarized in the following table

Disease/other tests	Test	Sample	Collection vial	Tested by
Brucellosis	ELISA	Serum	Serum vacutainer (Red cap)	CDDL/RDDL/ NDDB-CALF
IBR	ELISA	Serum	Serum vacutainer (Red cap)	CDDL/RDDL/ NDDB-CALF
TB	Delayed Type Hypersensitivity (DTH) Test using PPD (IVRI)	Intra-dermal	NA	Project Vet
JD	DTH Test using Johnin (IVRI)	Intra-dermal	NA	Project Vet

General male calf procurement protocols

- 1) Male calves born out of nominated mating shall be procured as soon as farmers agree to handover the calves (maybe even before three months of age but after 3 days).

- 2) The calf shall be physically examined for general health and physical deformities, like cryptorchid/monorchid, inguinal hernia, blindness etc, and only normal calves procured.
- 3) Testing is to be done by approved laboratories like CDDL, RDDDL and NDDDB-CALF.
- 4) Additional numbers of tests are not mandatory but would greatly increase the chances of disease detection.
- 5) The procedure of selection and lifting of male calf by the project and terms of payment to all concerned farmers should be explained right at the time of insemination of dam with proven bull semen and again if a male calf is born.
- 6) Since Brucellosis vaccination is being carried out in PT/PS project areas, the vaccination status of the dams also need to be recorded irrespective of the result.
- 7) Parentage testing shall be done preferably before procurement of the calves. However in case of exigencies/ chances of loss of bull calves due to poor management, the testing shall be attempted after procurement preferably during pre-quarantine/ Quarantine.
- 8) All male calves (crossbred, exotic and indigenous) should be free from following conditions before shifting from quarantine:
 - a. Genetically transmitted diseases namely, BLAD, DUMPS, Citrullinaemia and Factor IX deficiency.
 - b. Chromosomal abnormalities.
- 9) In case of PS projects, calves may be procured from field (after they and their dams test negative to all MSP diseases) and maintained in the rearing centre. They may be shifted to quarantine station after again testing negative to all MSP diseases.

Calves when procured are below 3 months of age

- 1) Such calves should be reared separately at a pre-quarantine facility till they are screened for MSP diseases at the age of 2.5-3 months.

- 2) To be maintained in a pre-quarantine in isolation units till they attain 3 months of age.
- 3) They can be shifted to pre-quarantine even before tests are done on the dam or test results of the dam are pending.
- 4) In case the dam has been sold by the farmer, efforts should be made to trace/locate the dam. If the dam is not traceable, the calf shall not be procured.
- 5) To reduce the number of visits, the time of blood collection may be adjusted in such a manner that sufficient sample is available for both disease and parentage testing.
- 6) Calves in pre-quarantine are shifted to quarantine only if they and their dams are negative to TB & JD, Brucella & IBR (if IBR free policy is pursued) and their parentage is confirmed.
- 7) Calves positive to Brucella & IBR (if IBR free status is the policy) in pre-quarantine may be retained till they are 9 months of age and retested and only negative animals are shifted to quarantine.
- 8) The final test for each MSP disease must be conducted within the last 15 days while in pre-quarantine or quarantine.
- 9) The calves must be shifted from pre-quarantine to quarantine and from quarantine to rearing station within 15 days of receiving the results of the tests.

Calves when procured are above 3 months of age

They can be brought directly to the quarantine facility only when they and their dams are tested negative for TB, JD and Brucellosis. Calves also should be tested free for IBR (if IBR free herd policy is being pursued). The calves' parentage also should be confirmed before shifting to quarantine or during the quarantine.

Disease testing

The general norms for disease testing are summarized in the table below.

S.No	Disease	Time of testing	
		Dam testing	Calf testing
1	Bovine Tuberculosis (bTB)	Minimum 6 weeks post-calving.	Aged 2 months or above
2	Johne's Disease (JD)	Minimum 6 weeks post-calving.	Aged 2 months or above
3	Brucella	Minimum 21 days post-calving.	Aged 2 months or above, retested at 9 months if positive
4	Infectious Bovine Rhinotracheitis (IBR)	Minimum 21 days post-calving.	Aged 2 months or above, retested at 9 months if positive

Male calves are culled if either dam or its calf is positive to any of the tests, the exception being IBR (if an endemic herd is being maintained). Please see detailed protocol given under each disease for further clarifications.

2.1.1 Bovine Tuberculosis (bTB)

Name of test	Delayed Type Hypersensitivity Test
Reagent used	Bovine tuberculin PPD
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	<p>Positive : Increase in skin thickness of 4 mm or more, or presence of clinical signs viz. exudation, necrosis, pain, inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation.</p> <p>Negative : Increase in skin thickness less than 2 mm & without clinical signs viz. exudation, necrosis, pain,</p>

	<p>inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation.</p> <p>Inconclusive : Increase in skin thickness more than 2mm & less than 4mm, absence of above clinical signs, 72 hours post-inoculation.</p>
Testing at bull production programme villages	
Animals eligible for testing	<ol style="list-style-type: none"> 1. Dams of male calves are tested after a minimum of 6 weeks post calving but not exceeding 15 days before procuring the male calf. 2. Male calves above 2 months of age to be tested not more 15 days before shifting to quarantine.
Criteria for rejection of male calves above 2 months of age	<ol style="list-style-type: none"> 1. Male calf rejected if dam positive (even if calf tests negative) 2. Male calf rejected if positive
Criteria for selection of male calves above 2 months of age	<ol style="list-style-type: none"> 1. Dam of the selected male calf is TB negative 2. Calf is also TB negative.
Dams and calves with inconclusive result (>2 but <4mm increase in skin thickness)	<ol style="list-style-type: none"> 1. Take reading again at 96 hours post inoculation. 2. Repeat after 42 days if result still inconclusive. 3. Reject male calf if the repeat test of dam or its calf is again inconclusive. <p>Or;</p> <ol style="list-style-type: none"> 1. Reject the calf if either dam or its calf shows inconclusive result.

Testing in pre-quarantine	
Male calves eligible for testing	At the age of 2 months or above
Test schedule	One test towards the end of pre-quarantine (last 15 days)
Criteria for rejection of male calf	<ol style="list-style-type: none"> 1. Dam tested at minimum of 6 weeks post calving is positive.(Even if calf is negative) 2. The male calf is positive when tested at 2 months or above.
Male calves with inconclusive result (>2 but <4mm increase in skin thickness)	<ol style="list-style-type: none"> 1. Take reading again at 96 hours post inoculation. 2. Repeat after 42 days if result still inconclusive. 3. Cull the calf if the repeat test is also inconclusive. <p style="text-align: center;">Or;</p> <ol style="list-style-type: none"> 1. Cull the calf
Criteria for shifting to quarantine	<ol style="list-style-type: none"> 1. Dam is negative 2. Calf is negative
Testing in Quarantine/Rearing Centre(PS)	
Animals eligible for testing	All male calves in quarantine/rearing centre
Test schedule	Two tests with a minimum interval of 42 days between the tests, the last being within the last 15 days of quarantine/rearing centre.
Male calves with	<ol style="list-style-type: none"> 1. Take reading again at 96 hours post inoculation. 2. Repeat after 42 days if result still inconclusive.

inconclusive result (>2 but <4mm increase in skin thickness)	<p>3. Cull the calf if repeat test is also inconclusive. Or;</p> <p>4. Cull the calf</p>
Positive calf	Culled

2.1.2 Johne's Disease (JD)

Name of test	Delayed Hypersensitivity Test
Reagent used	Johnin
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	<p>Positive : Increase in skin thickness of 4 mm or more, or presence of clinical signs viz. exudation, necrosis, pain, inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation.</p> <p>Negative : Increase in skin thickness less than 2 mm & without clinical signs viz. exudation, necrosis, pain, inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation.</p> <p>Inconclusive : Increase in skin thickness more than 2mm & less than 4mm, absence of above clinical signs, 72 hours post-inoculation.</p>
Testing at bull production programme villages	

Animals eligible for testing	<ol style="list-style-type: none"> 1. Dams of male calves are tested after a minimum of 6 weeks post calving but not exceeding 15 days before procuring the male calf. 2. Male calves above 2 months of age to be tested not more 15 days before shifting to quarantine.
Criteria for rejection of male calves above 2 months of age	<ol style="list-style-type: none"> 1. Male calf rejected if dam positive (even if calf tests negative) 2. Male calf rejected if positive
Dams and calves with inconclusive result (>2 but <4mm increase in skin thickness)	<ol style="list-style-type: none"> 1. Take reading again at 96 hours post inoculation. 2. Repeat after 42 days if result still inconclusive. 3. Reject male calf if the repeat test of dam or its calf is again inconclusive. <p>Or;</p> <ol style="list-style-type: none"> 1. Reject the calf if either dam or its calf shows inconclusive result.
Criteria for selection of male calves above 2 months of age	<ol style="list-style-type: none"> 1. Dam of the selected male calf is TB negative 2. Calf is also TB negative.
Testing in pre-quarantine	
Male calves eligible for testing	At the age of 2 months or above
Test schedule	One test towards the end of pre-quarantine (last 15 days)

Criteria for rejection of male calf	<ol style="list-style-type: none"> 1. Dam tested at minimum of 6 weeks post calving is positive.(Even if calf is negative) 2. The male calf is positive when tested at 2 months or above.
Male calves with inconclusive result (>2 but <4mm increase in skin thickness)	<ol style="list-style-type: none"> 1. Take reading again at 96 hours post inoculation. 2. Repeat after 42 days if result still inconclusive. 3. Cull the calf if the repeat test is also inconclusive. <p>Or;</p> <ol style="list-style-type: none"> 1. Cull the calf
Criteria for shifting to quarantine	<ol style="list-style-type: none"> 1. Dam is negative 2. Calf is negative
Testing in Quarantine/Rearing Centre (PS)	
Animals eligible for testing	All male calves in quarantine /rearing centre
Test schedule	Two tests with a minimum interval of 42 days between the tests, the last test being during the last 15 days in quarantine.
Male calves with inconclusive result (>2 but <4mm increase in skin thickness)	<ol style="list-style-type: none"> 1. Take reading again at 96 hours post inoculation. 2. Repeat after 42 days if result still inconclusive. 3. Cull the calf if repeat test is also inconclusive. <p>Or;</p> <ol style="list-style-type: none"> 4. Cull the calf
Positive calf	Culled

2.1.3 Brucellosis

Name of test	Enzyme Linked Immunosorbant Assay (ELISA)
Sample required	Serum
Testing at bull production programme villages	
Animals eligible for testing	<ol style="list-style-type: none"> 1. Dams of male calves after a minimum of 21-30 days post calving but not exceeding 15 days before procuring the male calf. 2. Male calves above 2 months of age. 3. Male calves are tested not more 15 days before shifting to quarantine.
Criteria for rejection of male calves above 2 months of age	<ol style="list-style-type: none"> 1. Male calf rejected if dam positive. 2. Male calf above 9 months of age rejected if positive.
Criteria for selection of male calves above 2 months of age	<ol style="list-style-type: none"> 1. Dam of the selected male calf is brucella negative.
Testing in pre-quarantine	
Male calves eligible for testing	At the age of 2 months or above
Test schedule	One test towards the end of pre-quarantine (last 15 days).
Criteria for	<ol style="list-style-type: none"> 1. Dam tested at minimum 21-30 days post calving

rejection of male calf	is positive.
Criteria for shifting to quarantine	<ol style="list-style-type: none"> 1. Dam is negative. 2. Male calf above 9 months is negative. (<i>Male calves aged 2 months and above which are positive are retained in pre-quarantine in isolation till they attain 9 months</i>)
Testing in Quarantine /Rearing Centre (PS)	
Animals eligible for testing	All male calves in quarantine/rearing centre
Test schedule	Two tests with a minimum interval of 30 days between the tests, the last being within the last 15 days of quarantine.
Positive calf	Culled if above 9 months of age

2.1.4 Infectious Bovine Rhinotracheitis (IBR)

Name of test	Enzyme Linked Immunosorbant Assay (ELISA)
Sample required	Serum
Testing at PT villages	
No testing carried out at village level	
Testing at pre-quarantine	
Male calves eligible for testing	At the age of 2 months or above
Test schedule	One test towards the end of pre-quarantine (last 15 days)
Male calves (In	Moved to quarantine irrespective of IBR positivity but

endemic herds)	negative for other MSP diseases.
Male calves (while pursuing IBR free herd)	Positive animals above 9 months of age culled (<i>Male calves aged 3 months and above which are positive are retained in pre-quarantine in isolation till they attain 9 months)</i>)
Testing in Quarantine/Rearing Centre (PS)	
Male calves eligible for testing	All male calves in quarantine /rearing centre
Test schedule	Two tests with a minimum interval of 30 days between the tests, the last being in the last 15 days of quarantine/rearing centre.
Male calves (In endemic herds)	One testing done to know its status. Positive animals retained and moved to rearing station.
Male calves (while pursuing IBR free herd)	Positive animals culled if above 9 months of age.

3 Health guidelines

The health guidelines have been prepared keeping in mind the general problems encountered in the field that causes infection and disease in calves which often lead to loss of the animal, especially in case of crossbreds. Though it is not mandatory to follow the guidelines, practicing it would greatly reduce the chances of calves succumbing to various diseases that are prevalent in our country.

General practices

S.N	Practice	Field	Pre-Q	Qua	Remarks
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1	Dipping (not merely swabbing) of navel with Tr.Iodine	To be done immediately after birth and followed 12-24 hrs later. Farmers may be provided with Tr.iodine (minimum 7%) beforehand.	-	-	Greatly reduces chances of serious infections like sepsis/navel/joint ill.
2	Colostrum feeding	Farmer to be educated on the time of feeding and quantity to be fed.	-	-	Greatly reduces the chances of the animal succumbing to infections.
3	Checking the health of the animal	Check the animal for signs of joint ill/navel ill/diarrhoea, general health etc			Avoid procuring animals that are unthrifty or unhealthy and showing signs of joint ill/navel ill to the Pre-Q or Qua.
4	Transportation to Pre-Q	Proper bedding to be provided. Tie a cloth band around the waste tightly supporting the umbilicus.	Tie a cloth band around umbilicus in cases of herniation.		Tying a cloth band around the waste reduces the occurrence or increases cure rate of umbilical hernia post transportation or while in Pre-Q.
5	Weight monitoring	-	Daily/weekly weight monitoring		Minimum weight gain of 500 g per day for CB and 400g per day for Indigenous breeds and Buffaloes.
6	Temperature Monitoring	-	Twice daily		Immediate intervention in case of variation.
7	Body condition monitoring	-	Hydration levels to be checked by skin test on a daily basis especially during		Appropriate measures to be taken to rehydrate (i/v and oral) and to

			scouring.	correct acidosis and hypoglycaemia.
8	Cleaning and disinfection of pens	-	Twice weekly and before and after animals are quartered	Twice weekly and before and after animals are quartered
9	Testing of calf starter/feed/milk replacer		Testing for nutrient content / aflatoxins/coliforms.	Every fresh batch procured may be tested before usage.

Vaccination

The following vaccinations may be carried out as indicated in the table:

S.No	Disease*	Eligible calves	Remarks
1	Foot and Mouth Disease (FMD)	4 months and above	Booster after 1 month
2	Haemorrhagic Septicaemia (HS)	6 months and above	In endemic areas only, a month before expected occurrence of the disease
3	Black Quarter (BQ)	6 months and above	
4	Anthrax	2 months and above especially when exposed to grazing	In endemic areas only, a month before expected occurrence of the disease.
6	Theileriosis	3 months and above	Only for cross bred and exotic animals

**The route and dosage recommended by the manufacturer to be followed*

Disease prophylaxis

The following protocol may be adopted based on the disease prevalent in the region/station.

S.No	Disease	Eligible calves	Prophylaxis			Remarks
			Field	Pre-Q	Quarantine	
1	Theileriosis	All age groups	Single dose of Buparvaquone @ 2.5 mg/Kg BW I/M not exceeding a week before	Single dose of Buparvaquone @ 2.5 mg/Kg BW I/M not exceeding a week before	Single dose of Buparvaquone @ 2.5 mg/Kg I/M during last 15 days of Qua.	Only to be done in exotic and crossbred animals.

			lifting	shifting to Qua.		
2	Anaplasmosis	All age groups	Single dose of long acting oxytetracycline-@20 mg/Kg BW I/M not exceeding a week before lifting.	Single dose of long acting oxytetracycline-@20 mg/Kg BW I/M not exceeding a week after the previous dose given in field.	Two doses of long acting oxytetracycline-@20 mg/Kg BW I/M at an interval of 1 week, during last 15 days of Qua.	Four doses of Oxy (LA) at 3 day interval @ 20 mg/Kg BW I/M have greater chances of eliminating carrier stage in anaplasmosis. OR Imidocarb dipropionate @ 5mg/Kg I/M in two doses at 14 day interval may eliminate anaplasmosis infection.
3	Leptospirosis	All age groups	Single dose of Isometamidium Chloride (1-2mg/Kg BW) I/M or Quinapyramine (5 mg/Kg BW) I/M not exceeding a week before lifting.	Single dose of Isometamidium Chloride (1-2mg/Kg BW) or Quinapyramine (5 mg/Kg BW) not exceeding a week before shifting to quarantine.	Single dose of Isometamidium Chloride (1-2mg/Kg BW) I/M or Quinapyramine (5 mg/Kg BW) I/M during last 15 days of Qua.	In high prevalent areas, a prophylactic dosage may also be given at the onset of monsoon.
4	Trypanosomiasis	All age groups	-	Amprolium @ 5mg/Kg BW/day orally for 21 days from the date of arrival.	Amprolium @ 5mg/Kg BW/day orally for 21 days from the date of arrival.	Need to be done only if coccidiosis is a problem.
5	Coccidiosis	Up to 1 year				

Tick control

The following protocol may be adopted for control of ticks.

Type of acaricide*	Field	Pre-Quarantine	Quarantine	Eligible animals
Injectable endo-ectoparasiticide Or;	One dose 1-2 weeks before lifting	One dose not exceeding one week before shifting to quarantine	(a) One dose 7-14 days of quarantine (keeping an interval of 2-3 weeks from the previous treatment) (b) One dose at last 15 days of quarantine	All age groups

Topical acaricide	One application not exceeding 2 days before lifting	One application not exceeding 2 days before shifting to quarantine	(a) One application immediately on arrival at quarantine (b) One application at last 15 days of quarantine	All age groups
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Worm control

The following protocol may be adopted for helminth control

Type of anthelmintic	Pre-Quarantine	Quarantine	Eligible animals
Oral Or;	(a) One dose of a broad spectrum anthelmintic immediately on arrival at pre-quarantine. (b) One dose of an oral broad spectrum anthelmintic 3-4 weeks after first dose but not exceeding 6 weeks. (c) An oral broad spectrum anthelmintic not exceeding one week before shifting to quarantine.	(a) A broad spectrum anthelmintic immediately on arrival at quarantine(0 day) (b) A broad spectrum anthelmintic between 20-30 days of quarantine (c) A broad spectrum anthelmintic not exceeding one week before shifting from quarantine or one week before any vaccination.	All age groups
Injectable ecto-endo parasiticide (EEP)	Injectable EEP 1-2 weeks after arrival at pre-quarantine (keeping an interval of 2-3 weeks between two treatments	Injectable EEP at 7-14 days of quarantine (keeping an interval of 2-3 weeks from the previous treatment) (b) Injectable EEP at 100-110 days of quarantine.	All age groups – May be synchronized with tick control protocol.