F.No.3-13/2012-AHT(NPCBB)
Government of India
Ministry of Agriculture
Department of Animal Husbandry, Dairying & Fisheries

Krishi Bhawan, New Delhi.
Dated 6th June 2012.

Subject: Implementation of Minimum Standard Protocol (MSP) for frozen semen production in Semen Stations (FSBS) – regarding.

A minimum standard Protocol for semen Production was developed after consultations with the experts and circulated among all the agencies having bovine frozen semen production facilities vide letter No. 55-14/2004-AHT dated 20.5.2004. The document was revised and circulated among all the agencies vide letter dated 29.4.2005. During the last evaluation and initiation of NDP-I it was felt that there is need to revise the existing document in order to improve quality of semen production in the country.

2. The revised MSP has been developed after discussions with the experts from State Animal Husbandry Departments, BAIF, ICAR, NDDB, Milk Federations and DADF during the meeting held at NDDB Anand. Comments/ suggestions received from agencies involved in semen production are also incorporated in the document.

3. Revised MSP is enclosed herewith as ready reference. It is requested that the document may be given wide publicity and recommended for adoption by all concerned agencies in the State. During the next evaluation semen stations will be evaluated on the basis of revised MSP.

(R.S. Jayal)
Under Secretary to Government of India

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Minimum Standards

For
Production of Bovine
Frozen Semen

PROPOSED MINIMUM STANDARDS FOR PRODUCTION OF BOVINE FROZEN SEMEN

Artificial Insemination with frozen semen has been proved to be the best tool world wide for genetic improvement through dissemination of superior germplasm. This objective can be achieved only if the frozen semen used in AI programme conforms to the quality standards. For production and distribution of quality semen, it is most important that the bulls used in AI programme satisfy quality norms, bulls are disease free and semen is harvested and processed in accordance with the standard protocols. The least protocols required for production of quality semen are covered in this manual. Failure to observe these guidelines could lead to production of poor quality semen making it unfit for distribution to AI centres.

1. Standard for Genetic Merit of Breeding Bulls

Bulls procured should be the ones produced following prescribed Minimum Standard Protocols and Standard Operating Procedures for Progeny Testing (PT) through Government approved Progeny testing (PT) Programmes. If such bulls are not available and if there are no PT programmes for certain breeds, the procurement of bulls should be based on the dam's Standard lactation yield. Breed wise dam's lactation yields are given below. Preferably, the Lactation yield would be arrived at by recording the animal once a month continuously for 11 times or until the animal becomes dry. Standard Lactation Yield of the milk recorded animal should be calculated using the Test Interval Method (A4) described at Section 2.1.5.1 of the International Agreement of Recording Practices published by International Committee for Animal Recording (ICAR).

Breed	Dam's Lactation yield (Kgs)		
	First	Best	Fat %
Pure HF	4500	5600	3.5
Pure Jersey	3000	3750	5.0
Sahiwal	2400	3000	4.0
Red Sindhi	2000	2500	4.5
Gir	2400	3000	4.5
Kankrej	2000	2500	4.5
Tharparkar	2000	2500	4.0
Hariana	1600	2000	4.0
Rathi	1600	2000	4.0
Ongole	1100	1600	4.0
Deoni	800	1000	4.0
Khillar	380	500	4.0
Dangi	400	530	4.0
Amritmahal	400	500	4.0
HFCross- F2	4000	5000	4.0
Jersey CB- F2	2800	3500	4.5
Sunandini	2500	3000	3.5
Murrah	2400	3000	7.0
Mehsana	2400	3000	7.0
Nili Ravi	2400	3000	7.0
Jaffrabadi	2800	3500	8.0
Surti	1600	2000	7.0
Banni	2400	3000	7.0
Bhadawari	1300	1600	8.0
Pandharpuri	1300	1600	7.0

Dam's milk yield for F1 crosses will be as that of the indigenous dam's i.e. Gir, Sahiwal, Kankrej, Red Sindhi, etc.

For import of bulls and embryos, the standards for import of germplasm as prescribed in the "Guidelines for export / import of bovine germplasm" issued by DADF, MoA, GoI and as revised from time to time shall be followed.

2. Physical Examination

Before procuring new bull calves/bulls for a semen station, a thorough physical examination shall be conducted by an accredited Official / Veterinarian to ensure that the bulls are free from abnormality and do not display clinical symptom(s) of any infection or any contagious diseases.

Standards for scrotal circumference and weight gain index for various breeds shall be fixed by initiating age wise recording of scrotal circumference once in three months and body weight once a month, by the semen stations. For every new calf procured, the measurement of scrotal circumference and body weight should be initiated immediately.

Prior to introduction of new bulls for semen collection, breeding soundness examination shall also be carried out.

3. Karyotying and testing for genetically transmitted diseases

It is necessary that all animals be karyotyped to rule out any chromosomal defects. Specific tests may also be conducted for genetically transmitted diseases as given in the table:

Breed	**Tests to be carried out**
Indigenous cattle and buffaloes	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion

	Deficiency (BLAD), Citrullinemia
HF and HF crossbreds	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia, Deficiency of Uridine Monophosphate Synthase (DUMPS)
Jersey and Jersey Crossbreds	Factor XI deficiency syndrome, Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia

4. Quarantine

A quarantine period of **minimum 60 days*** is compulsory before bringing new bulls into a semen station. Only after favourable results from the health control point, the bulls shall be admitted to the semen station. Relevant definitions are given in Annexure- 1

a) In the quarantine station, new animals shall be housed for a minimum of **60** days in a place which is effectively separated and away from (preferably at a distance of 5 km) the facilities occupied by resident bulls. Manpower deployed and all equipment used in handling, feeding, watering and cleaning the new bulls shall not be shared with the resident herd(s).

b) Each new animal in quarantine station will be tested against major contagious diseases before its entry to resident herd e.g. TB, JD, Brucellosis, Campylobacteriosis and Trichomoniasis. All tests shall be done by an accredited agency or disease diagnostic laboratory as indicated in Annexure- 2.

c) During quarantine period, the bulls shall be vaccinated against FMD, HS, BQ, Theileriosis and Anthrax. However, vaccinations against bacterial diseases shall be done only if there is an outbreak or prevalence of a particular disease.

Once the quarantine period is over, all bulls shall be introduced to the young bull rearing station.

**The procedure and duration for quarantine in different situations is given in Annexures- 3A, 3B, 3C & 3D.*

5. Testing of Bulls

Testing protocols for bulls against Tuberculosis, Johne's disease, Brucellosis, Campylobacteriosis and Trichomoniasis are given in Annexures- 4 to 8. As per OIE guidelines, the breeding bulls should be free from above mentioned diseases. Though Johne's disease is not a sexually transmitted disease but from the herd health point of view, bulls found positive should be removed and therefore it has been included in the MSP. The bulls in the rearing station and the resident herd should go through periodical testing and vaccinations as per the schedule listed in the manual.

6. Vaccination Schedule

The bulls shall be vaccinated against FMD, HS, BQ, Theileriosis and Anthrax. However, vaccinations against bacterial diseases shall be done only if there is an outbreak or prevalence of a particular disease.

Theileriosis – Exotic and crossbred bulls shall be vaccinated once in their lifetime.

To reduce lay off time, the bulls shall be vaccinated on the rest day or the day after completing semen collection. Sexual rest may not be required unless otherwise febrile condition is noticed.

The semen station shall arrange for carrying out ring vaccinations for all cloven footed animals including swines against FMD within a radius of 10 km around the semen station. Vaccinations against HS and BQ shall be carried out in the areas having incidence of these diseases.

7. Culling of Bulls and Semen Doses due to Specific Diseases

Diseases	**Bulls**	**Semen doses**
FMD	Retain	Last one month's doses to be discarded, refer Annexure- 9
Brucellosis	Castrate & remove	FS doses in stock to be discarded since the last negative test
TB	Remove	FS doses in stock to be discarded since the last negative test
JD	Remove	FS doses in stock to be discarded since the last negative test
Campylobacteriosis	Treat and retain	FS doses in stock to be discarded since the last negative test
Trichomoniasis	Treat and retain	FS doses in stock to be discarded since the last negative test

The semen station must remove bulls (within 48 hours) which are positive for Brucellosis, TB and JD. Bulls found positive for Campylobacteriosis and Trichomoniasis shall be isolated and treated. Besides, the semen station shall cull those bulls which have

completed eight years of productive period or 3 lakh semen doses, whichever is achieved earlier. In addition, the bulls with poor libido, poor semen quality, incurable lameness, etc. shall also be culled.

8. Housing

Bull sheds shall have spacious individual pens with adequate loafing area, manger and water trough with access to drinking water all time. Adequate shade around the bull shed shall be provided. The roof shall be made of asbestos or suitable materials. During summer, cooling system with sprinklers and fans is required particularly for the buffaloes and exotic bulls. Disinfectants like **formalin or phenyl** based compounds **shall not be used** in the bull sheds. Alternatively, compounds containing Gluteraldehyde shall be used. Weekly spraying of Sodium Carbonate (4%) solution shall also be practiced. The floor should be sterilized at least once a year by a blowlamp or by burning straws. At one corner of the farm, there shall be an isolation shed for separating ailing / sick bull(s) for treatment. Bull(s) once diagnosed suffering from infectious diseases shall be removed immediately from semen station for safety of other bulls.

There should be separate staff and separate bio-security arrangements for semen station and female herd, if any.

9. Management of Bulls

The objective of daily care of bulls is to ensure a satisfactory state of cleanliness. For proper management of bulls, the following points shall be considered:

a) The bulls shall be kept under hygienic conditions at all times.

b) The coat of the bulls shall be kept clean and generally short. The hooves shall be regularly trimmed.

c) The length of the tuft of hairs at the preputial orifice, which is invariably soiled, shall be cut to about 2 cm. The hair would not be removed altogether, because of its protective role. If cut too short, it may cause irritation of the preputial mucosa.

d) Bulls shall be brushed and groomed regularly, and where necessary, special attention shall be given to the underside of the abdomen, a day prior to semen collection.

e) Cleaning of the prepuce with sterile normal saline solution may be done every ten days if the microbial load is within the prescribed limits. Cleaning prior to the day of collection can be practiced if the microbial load in frozen semen is beyond the prescribed limit.

f) In the event of obvious soiling, careful cleaning of the preputial orifice and the adjoining areas with soap or a detergent is recommended; followed by thorough rinsing and drying.

g) Scientific feeding schedule shall be followed for the bulls. A general guideline is attached as Annexure- 10. Semen station shall carryout routine quality analysis of feed and fodder for arriving at a balanced ration.

10. Semen Collection

a) Ideally, the floor of the collection yard shall be made of concrete layer at a depth of one foot from the ground level. Mixture of sand and limestone shall be used to fill up to ground level and

pressed firmly. If it is not possible to renovate the entire collection arena, at least the mounting area shall have sand and limestone mixture for proper footing of bulls. Alternatively, good quality rubber mat (with interlocking arrangement) or coir mat shall be put into concrete groove of the mounting area for adequate cushioning effect. After collection, the area must be thoroughly cleaned and odorless disinfectant solution (Colloidal iodine) be sprayed. A dusty floor shall be avoided to prevent dust falling on the AV / semen samples.

b) On the day of collection, before collecting semen, the bulls shall be properly washed and cleaned. After that, the prepuce shall be cleaned externally with normal saline and a sterilized paper napkin or sterilized cloth napkin soaked in normal saline to remove any sand or dust particles. For each bull a separate napkin shall be used.

c) The person responsible to carry out preputial wash must use disposable gloves and separate sterilized nozzle for each bull to avoid transmission of infection from one bull to another.

d) Semen collection should be individualized based on the bull

e) Sexual preparation (number of false mounts and restraint) of the bulls may be done considering the individual behavior of the bulls and not generalized. For this purpose, the sexual behavior of the individual bulls shall be studied and documented

f) As a general rule, bulls shall be sexually prepared by giving two / three false mounts followed by restraint. The gap between two ejaculates shall be half an hour to one hour depending on the

bull. Second ejaculate shall be taken with proper preparation of bulls.

g) Sterilized bull aprons shall be used to avoid penis touching hindquarter of the dummy.

h) Before every collection, the semen collector shall either wash his hands with 0.1% Savlon solution or use disposable gloves or do both. The semen collector shall not touch the penis.

i) Preferably veterinarians shall take semen collection. If semen is collected by staff, a veterinarian shall remain present to supervise the collection process. While taking collection, it shall be ensured that AV is not thrust on penis of bull, instead penis should be guided to AV.

j) Immediately after collection, the AVs shall be thoroughly cleaned by non-spermicidal neutral detergent. Separate AVs shall be used for each ejaculation. The AV shall be changed even if the bull has inserted its penis without successful ejaculation. The same AV shall not be used twice. The AVs shall always be kept inverted and the collection tube shall be covered with felt / water jacket (plastic bottle filled with warm water at 34^{o} C) to avoid cold shock. The open end of sterilized AVs shall be covered with aluminium foil, which would be removed at the time when bull is ready for giving semen.

k) Appropriate size AVs, ranging from 8-14", shall be used for cattle and buffaloes to ensure semen is ejaculated in cone. For buffaloes, goat AVs can also be used. The cone shall be of top quality Neoprene rubber.

l) Use of lubricant shall be avoided. If it is extremely essential to use lubricant, separate sterilized glass rods shall be used for smearing K-Y Jelly on each AV.

m) The AV shall not to be shaken after ejaculation; otherwise lubricant and debris may mix with the semen samples.

n) As soon as the first ejaculate is taken, the bull apron should be removed and dipped in the plastic tub filled with detergent lotion. For second ejaculate, a fresh apron should be tied to the bull

o) The entry of visitors and staff / labourers (other than those not involved in semen collection) shall be strictly prohibited in the collection arena at the time of semen collection.

p) Protective clothing (barn coat) and gumboots shall be used by the veterinarians and personnel during semen collection. Gumboots and barn coat should be washed immediately after completion of semen collection work.

q) Semen stations must follow the norm of minimum two ejaculates per collection and minimum two collections per bull per week for taking at least 90 collections and 180 ejaculates annually from each adult bull. However, a maximum number of collections per bull would depend on the individual capacity of the bull.

11. Handling, processing & freezing of semen

11 (A) Premises

a) Sufficient trees shall be planted and lawns prepared around the semen station to reduce dust.

b) The ceiling and walls of the laboratory shall be made up of non-porous materials. All cracks and crevices shall be sealed to control pests and insects.

c) Entry of persons to the laboratory, other than laboratory personnel, shall be strictly restricted. Airlock system or anti-room shall be provided to avoid direct entry to the semen-processing laboratory.

d) Laboratory windows shall preferably be made of double sheet glass with fixed aluminium frame. The glass panes shall be plastered with sun control films to avoid direct sunlight. The doors shall be kept closed, especially during dilutor preparation and semen processing.

e) Preferably cassette type or, split type air conditioners fitted with air purifying system with remote temperature control mechanism should be installed to maintain the room temperature at 20°C - 22°C. The number of ACs to be fixed to sustain this temperature shall depend on the size of the processing room. Maintaining this temperature is most important to achieve the best results when single step dilution method is followed for freezing semen. The flow of air from AC must not be towards the front side of the Laminar Air Flow Unit. Adequate number of thermometers shall be kept in a few places in the laboratory to check the room temperature.

Alternatively, central cooling with 10 to 15 air exchanges should be fixed, especially for the semen processing

laboratory. This helps to control the bacterial load in the semen-processing laboratory and in removing obnoxious odour. The processing laboratory should ideally maintain around 55% relative humidity.

f) Sink drains shall be decontaminated routinely with a disinfectant. Sink shall not be placed in the semen processing room.

g) The floors shall be preferably made up of vitrified tiles. Floors and horizontal surfaces shall be cleaned and mopped with a disinfectant solution, as dirt and dust, which settle on these surfaces, are the main sources of contamination.

h) Unwanted furniture, equipment and materials shall not be kept in the laboratory as they only provide additional area for dust and spores to collect.

i) Appropriate number of germicidal UV lights (2470 A) with respect to area of laboratory, laminar airflow unit, apron and laboratory footwear cabinet may be fixed with a common operating switch outside the laboratory. These lights shall be switched 'on' at least 8 hours prior to commencement of work in the laboratory and shall be switched 'off' before beginning work. The date of installation of the UV lights shall be noted to facilitate replacement as the life of UV tube is of 2000 hours. A logbook should be maintained for timely replacement of UV lights.

j) The laboratory shall be fumigated twice a week with **Cold Fumigant**, using humidifier.

k) Fumigation should be supported by monitoring laboratory environment by bacterial load test. The bacterial load shall be measured every week to monitor pollution of the laboratory atmosphere.

l) The work platform, the parts of equipment and other items to be handled during processing of semen, shall be cleaned with 70% alcohol or Glutaril (Qualigen). It is advisable to repeat cleaning schedule after completing processing of semen.

m) Clean laboratory footwear, apron, hand gloves, mask and caps shall be compulsorily put on while working in the laboratory.

n) Eating, drinking, smoking, etc. shall be prohibited in the laboratory and unnecessary conversation should be discouraged. Besides, entry of persons shall be strictly restricted.

o) Long exposure of semen to ultraviolet rays, visible light in direct sunlight and white florescent light causes chromosomal damage and hence, direct exposure to such sources of light shall be avoided. Hence, there shall be provision for indirect or diffused lighting inside the semen processing room. Care shall also be taken not to switch on tube lights in CH cabinet and laminar air flow unit (LAFU). However, at the time of filling and sealing of straws in LAFU, diffused light could be used.

11 (B) Equipment

a) The exteriors of all equipment and furniture shall be cleaned weekly. The equipment shall be kept covered by plastic covers when not in use.

b) The pre-filter of Laminar Airflow unit shall be cleaned weekly. Routine servicing and DOP testing twice a year will ensure efficiency of HEPA filters. Alternatively, culture plate test shall be carried out at frequent interval to assess bacterial load of the air passing through the filters.

c) Digital photometer / Computer aided Spectrophotometer shall be validated with Haemocytometer readings for sperm concentration twice a year separately for cattle and buffalo (20 samples each).

d) The automatic semen straw filling and sealing machine shall be thoroughly cleaned, immediately after use.

e) The microscope lens shall be gently cleaned daily with a piece of cotton soaked in a mixture of ethyl and methyl alcohol (1:1) or a mixture of 80% ethyl alcohol and 20% ether)

f) The bio-freezer shall be defrosted and thoroughly cleaned and dried, immediately after use.

g) Incubators to maintain artificial vagina shall be cleaned and disinfected with 70% alcohol.

h) Single distilled water shall be used in autoclave and thermo-controlled water bath. The water bath shall be cleaned and filled with single distilled water on a regular basis.

i) The thermometer kept immersed in water bath shall be cleaned daily to have precise temperature reading or water bath fitted with digital display temperature indicator should be used.

j) The Liquid Nitrogen containers returned / received from foreign countries and contagious disease prone areas shall be disinfected thoroughly with 4% soda solution and finally with 1 to 4% formaldehyde.

k) The refrigerator meant for storing eggs, antibiotics and buffer shall not be used for storing vaccines and other materials. All such materials shall be stored at a place away from semen laboratory. The refrigerator used for storing eggs, etc. shall be sterilized every week using alcohol swab.

l) The following equipment should be validated by NABL certified laboratories:

i. Standard Thermometer
ii. Water Bath
iii. Weighing Balance
iv. Incubator
v. Autoclave
vi. Hot Air Oven
vii. Slide Warmer
viii. Micropipettes
ix. pH Meter

The following equipment calibration needs to be certified by Manufacturer/supplier:

i. Cold Handling Cabinet
ii. Laminar Air Flow Units
iii. Biological Freezer
iv. Microjet Ink Printer
v. Filling & Sealing Machine
vi. Photometer
vii. Triple distillation unit, etc;

m) All equipment used in semen processing should be covered under Annual Maintenance Contracts.

11 (C) Personnel Hygiene

Clothing, skin and hair of laboratory personnel are the sources of contamination. Hence, all should wear laboratory aprons and footwear all the time while they are in the laboratory. Hands shall be washed with soap and water and rinsed with 70% alcohol, before commencing work in the laboratory. The bull attendants must undergo test for TB every year**.** Other staff working in farm should be tested for TB once in two years. Restricted entry inside the semen processing room and freezing room shall be strictly adhered to.

11 (D) Diluents

a) Buffer and diluents should be prepared in a separate classified zone.

b) All disposable and reusable supplies coming in contact with the semen and dilutor must be sterile and devoid of toxins and pyrogens.

c) Prolonged storage of purified water is not recommended because water purity deteriorates progressively over a period of time as heavy metals leach from some glass and plastic storage vials / containers.

d) Glass ware, collection tubes, etc. shall not be handled from their rim / mouth.

e) Pipetting shall be done away with, instead, adjustable micropipettes and disposable tips shall be used.

f) After adding all the components of buffer viz. TRIS, Citric Acid, Glycerol and Fructose in double, preferably triple distilled water, it should be sterilized again. If buffer is prepared on the previous day and stored in the refrigerator, then antibiotics are to be added next day in the morning after warming it at 34°C.

g) Antibiotics in diluents: A combination of Penicillin and Streptomycin shall be used in diluents. However, it is better to use a combination of Gentamycin, Tylosin and Lincospectin (GTLS), if available, which can control Mycoplasma.

h) The eggs used for making dilutor must be **fresh**. The eggs shall be stored in refrigerator after wiping with dry cotton. Just before preparation of dilutor, eggs shall be wiped with 70% alcohol. To avoid Mycoplasma infection, eggs shall be purchased from known sources.

i) The required quantity of yolk shall be separated from albumin on sterile (autoclaved) standard filter papers (Whatman No.1/ Borosil) and yolk membrane shall be punctured using sterile glass rod, Pasteur pipette or sterile straws under the Laminar Air Flow Unit. Only fresh semen extender/dilutor shall be used because changes in the pH of stored extender are considered to be responsible for the deterioration of some nutrient components. Day old extender should not be used.

11 (E) Evaluation & Processing

a) The tube containing the freshly collected semen should be capped with aluminium foil as soon as it is placed in the pass box before transferring to the laboratory. The collection tube shall remain capped until processed.

b) As soon as the neat semen is received, it shall be kept in a thermo-controlled water bath at 34^{o} C under Laminar Air Flow Unit, after recording the volume of semen.

c) After examination of sperm concentration and initial motility, semen samples shall be primarily diluted with dilutor maintained at 34˚C.

After initial dilution of semen in the ratio of 1:1, the semen should be extended further after 7 minutes of cooling at 20˚C with dilutor maintained at the lab temperature. The semen samples should not get accumulated for long time in water bath, which may reduce their viability.

d) Sperm concentration shall be checked preferably by a digital photometer with auto dilutor, manufactured by a reputed

company. The photometer shall be calibrated separately taking 20 readings each for cattle and buffalo semen, at least once in six months, with haemocytometer readings. Semen samples showing less than 500 million / ml sperm concentration shall be discarded.

The volume of straws should be determined as it may vary from batch to batch. While determining the dilution rate as per the photometric reading, the actual volume of mini straw should be fed to the photometer. Straw volume of randomly drawn straws from a day's production should be checked as part of quality assurance and documented.

e) Semen samples selected for freezing should have minimum 70% initial progressive motility. Final dilution of semen, keeping a minimum of 20 million spermatozoa per dose, shall be done in appropriate flasks with the dilutor maintained at 34^{o} C.

f) Filling and sealing of semen shall be done under Laminar Air Flow Unit using sterile straws, filling nozzles and fresh rubber tubings. Rubber tubings shall be used once only. Reuse of rubber tubes is not recommended. Considering the advantages that French Mini Straws have over French Medium straws, the semen stations shall use French Mini straws.

g) Unused straws shall be repacked (air-tight) under Laminar Air Flow Unit before storage. Immediately after use, all the glass ware, rubber ware, plastic tips and other reusables shall be immersed in neutral detergent solution (to be kept in a plastic tub near the Laminar Air Flow Unit).

h) The freezing should be carried out as per the recommended protocols for freezing cattle and buffalo semen. After freezing gets over, the straws should be collected from the racks using scoop tongs. The operator should wear woollen gloves with leather gloves over it to avoid frost injury

11 (F) Colour Specifications:

All semen stations shall follow the following colour codes for filling of semen in straws:

Breed	**Colour**
Holstein	Pink/Rose
HF Crossbred	Pistachio Green (light green)
Jersey	Yellow
Jersey Crossbred	Salmon
Indigenous cattle	Orange
Sunandini	Blue
Buffalo	Grey

If any of above mentioned colour is not available, then transparent straws shall be used.

11 (G) Printing of Straws

Information pertaining to bull number, breed, name of the organization, year, batch number (as per the day of the year), ejaculate number, etc., shall be printed on straws, preferably after their filling and sealing. After printing, the ink gets instantly dried. If filled straws are printed and racked, the actual number of straws can be easily counted. While printing and racking, the room temperature shall be maintained at 20 ° C to 22 ° C**.**

All semen stations shall follow the following printing abbreviations:

Jersey	–	JY	Farm No. / Name
Holstein	–	HF	Breed
HF Cross	–	CB HF	Name of Institute
Jersey Cross	–	CB JY	Batch No. / Date of Prodn.
Sunandini	–	SUN	
Sahiwal	–	SAH	
Red Sindhi	–	RS	
Kankrej	–	KANK	
Gir	–	GIR	
Tharparkar	–	THAR	
Rathi	–	RATHI	
Hariana	–	HAR	
Ongole	–	ONGL	
Deoni	–	DEONI	
Khillar	–	KHLR	
Dangi	–	DANGI	
Amritmahal	–	AMHL	
Murrah Buffalo	–	MBF	
Surti Buffalo	–	SBF	
Jaffrabadi Buffalo	–	JBF	
Mehsana Buffalo	–	MSNB	
Nilli Ravi Buffalo	–	NLRVB	
Banni Buffalo	–	BBF	
Bhadawari Buffalo	–	BDBF	
Pandharpuri Buffalo	–	PNPB	

11 (H) Post thaw motility

After freezing, the semen straws shall be stored in a separate container. Post-thaw motility of semen should be examined at 24 hours (after freezing). Differences in observations shall be updated and recorded for the purpose of accepting a particular batch of semen doses. Whenever there is any doubt, post-thaw motility shall be examined by two experienced persons. Preferably, the person involved in evaluation of neat semen, shall not check the post thaw motility. For a minimum concentration of 20 million per dose, minimum acceptable post thaw motility shall be 50%. Semen doses below 50% progressive motility shall be discarded.

11 (I) Quality Checks for frozen semen

This includes (i) Quarterly testing of random samples from each batch for bacterial load using standard plate count (The standards for acceptable colony forming units (CFUs) in processed semen is 5000 per ml as per OIE norm. If the bacterial load exceeds the OIE limit, the semen doses are to be discarded.)

The frozen semen samples should not have uncountable CFUs as they may have pathogenic organisms. Therefore, semen showing crowded CFUs should be subjected to testing for pathogenic organisms by an outside laboratory.

(ii) Hypo osmotic swelling test (HOST) - for all bulls at least once in a quarter shall be mandatory (iii) Incubation test - for all bulls at least once in a quarter shall be mandatory (iv) Acrosome integrity test by Giemsa staining - for all bulls at least once in a quarter shall be mandatory. Alternatively, wet smear of semen shall be examined using DIC microscope (v) Percent Intact Acrosome - all

bulls to be covered once a quarter (vi) Sperm Concentration – randomly two samples per week each for cattle and buffalo.

A summary of quality tests to be conducted for frozen semen and their cut-off values are given in the following table:

Sr. No.	**QC Parameters**	**Cut- off Values**
1	Bacterial Load (FSD)	5000 CFUs /ml
2	Hypo Osmotic Swelling Test (HOST)	≥ 40%
3	Incubation / Thermo resistance Test	standard drop in motility by 10% after every 30 minutes
4	Acrosome Integrity (Fresh Semen)	≥ 70%
5	Percent Intact Acrosome (PIA)	≥ 65 %
6	Sperm Concentration	20 million spermatozoa per dose (0.25 ml Mini straw)

Validation of photometer shall be done once in 6 months by checking at least 20 samples each for cattle and buffalo. Neat semen shall be examined at an interval of every six months for morphological abnormalities, particularly for crossbred bulls. Morphological examination of sperms of young bulls must be carried out (at least six samples at weekly intervals) before introducing them in the herd. Semen should not be used if the sample contains a total abnormality of more than 20% and head and mid-piece abnormality (alone) of 7%.

Quality checking of semen straws, drawn randomly from the long storage containers once in three months, should be done as a part of quality assurance.

11 (J) Information System

In order to facilitate the information system, all the bulls maintained by the semen station must be identified by ear tags/ cold branding.

The semen stations shall use suitable software to record data pertaining to various activities and also should have online facility for the same. The semen stations producing more than one million doses may introduce software that can identify and trace the bulls and their ejaculates, production, storage and dispatch of semen (barcode system).

a) Volume of semen, density, motility, sperm concentration, dilution rate, total extended volume, post-thaw motility (24 hrs after freezing), and total number of doses produced, etc. shall be maintained. Pre-freeze and post-thaw motility shall be checked for new and problematic bulls.

b) Miscellaneous information regarding actual reason(s) for not donating semen, undesired percentage of gross morphological defects, semen pH, presence of dirt, dust, blood, pus, etc. in semen samples shall be noted and recorded.

c) Details of semen supplied to various agencies, including post-thaw motility at the time of dispatch, shall be recorded.

d) Fertility data of bulls, conception rate, records of the progeny associated with any genetic defect, percent male / female born, etc. shall be noted and recorded.

e) Report on microbiological examination of semen samples shall be maintained.

f) Record of all quality tests for neat and frozen semen samples shall be maintained.

11 (K) Semen Storage

To avoid accidental spread of diseases, the semen station shall follow the procedure of preserving semen doses for at least 30 days after production. Frozen semen doses produced at least 30 days prior to the date of dispatch should only be supplied for AI.

After checking post-thaw motility, if found acceptable, frozen semen doses shall be kept in temporary storage for 7 days. After temporary storage, the semen goblets shall be transferred to the bulk storage containers with proper recording of position in the canisters. After each dispatch, records redefining the position of remaining doses shall be updated.

Two reference samples of the doses dispatched to be drawn and retained for six months or a screen shot of randomly selected sample should be stored and a soft copy of which should be given to the customer

The goblets containing the semen should be well identified and precaution should be taken to see that each goblet has sufficient space for liquid nitrogen. Mini straws need special care and

should not be exposed above liquid nitrogen even for a short time (10 seconds) as they get warm faster and any exposure causes irreversible damage to sperm viability.

Liquid Nitrogen shall be replenished at regular intervals depending on the liquid nitrogen evaporation rate of the container.

12 Biosecurity

The risk of disease spread has grown manifold with increasing number of bulls maintained at the semen production center. With the expected higher risk, implementation of strict biosecurity measures at the semen stations assumes greater significance. Every semen station should have a well defined Biosecurity protocol put in place across all its activities.

13 Cleaning and Sterilisation

All the items to be washed shall be initially cleaned with running tap water and soaked in warm neutral detergent for at least 30 minutes. These items will then be thoroughly cleaned under running tap water using a brush. Filling nozzles shall be cleaned with pressure using 20 ml syringe. These materials shall be rinsed thoroughly with de-ionized water (5 to 7 changes) to completely remove detergent residues and other impurities. Appropriate procedure for sterilization of different materials, used in the semen station, is given below:

13.1 Laboratory and other areas

Cold fumigation solution is ideal for fumigation of laboratory and other areas. It should be done as per SOP.

13.2 Artificial Vagina (AV)

a) Cone from the AV and water from AV jacket shall be removed before washing.

b) Cones and AVs shall be cleaned thoroughly with a soft sponge brush under running tap water and then soaked in warm neutral cleaner for about 30 minutes, followed by proper rinsing in warm and clean water and then three times rinsing with double distilled water.

c) For sterilization, fully assembled AVs shall be autoclaved at 5 p.s.i. pressure for 20 minutes. During sterilization, the valve of AV shall be kept open. Alternatively, use AV sterilizer (using double distilled water in the sterilizer) for proper sterilization of AVs.

d) Finally AVs shall be stored overnight in an incubator at 45^{o} C.

e) To achieve best cleaning effect, AVs shall be cleaned immediately after use, preferably by non-spermicidal neutral detergent.

13.3 Glassware

a) The glassware shall be washed thoroughly with running tap water and soaked in warm, non-spermicidal neutral detergent solution for about 30 minutes.

b) Using appropriate nylon brush, the glassware shall be cleaned and rinsed with running tap water. The collection tubes shall be brushed at least 3 times and thoroughly cleaned and rinsed with distilled water.

c) Finally the glassware shall be rinsed three times with double distilled water and allowed to dry by keeping them inverted on a blotting paper or a drying stand made of SS/ plastic.

d) The open end/s of the dried glassware shall be covered with aluminium foil and sterilized in hot air oven at 160°C for one hour or at 180°C for 30 minutes. One item should be wrapped with newspaper and its mild charring will indicate proper sterilization.

13.4 Rubber wares

The washing and cleaning procedure of rubber wares is similar to that of glass ware. Care shall be taken to clean the rubber wares with sponge brush instead of nylon brush. Plastic tips shall be cleaned by water jet with force using a syringe. Sterilization technique, however, differs owing to the thermo-sensitivity of the rubber items. Thermo-resistant rubber wares shall be sterilized by autoclaving at 3 - 4 p.s.i. for 10 minutes. (**The rubber tubing for semen filling shall not be reused).**

13.5 Distilled Water

Fresh triple glass distilled water or Milli-Q purified water shall be autoclaved at 15 p.s.i. for 15 minutes and used for preparation of the dilutor.

13.6 Buffer

Buffer shall be sterilized by autoclaving at 5 p.s.i. pressure for 20 minutes. After autoclaving, buffer shall be cooled and stored in refrigerator.

13.7 Bacteriological Media

It is to be autoclaved at 15 p.s.i. pressure for 15 minutes.

13.8 Filter Papers

A bunch of clean filter papers of standard brand like Whatman No. 1 (thrashed to remove dirt, if any) shall be wrapped in thick cotton cloth for sterilization in an autoclave at 5 p.s.i. pressure for 20 minutes.

14 Summary of Sterilization

a) Autoclave

Sr.No.	Item	Pressure (p.s.i.)	Time (Min.)
1.	Artificial Vagina	5	20
2.	Buffer	5	20
3.	Plastic Tips	5	20
4.	Filter Papers	5	20
5.	Bull Apron	5	20
6.	Thermo-resistant Rubber wares	3-4	10

7.	Bacteriological Media	15	15
8.	Distilled Water	15	15
9.	Surgical Equipment	10	10

(The rubber wares can withstand above pressure and duration provided the quality is good)

b) Hot Air Oven

Sr.No.	Item	Temperature	Time (min.)
1.	Glass wares	160° C / 180° C	60/30
2.	Filling Nozzles	160° C / 180° C	60/30

c) AV Steriliser

Wherever Autoclave is not used, AVs and rubber cones shall be sterilised using AV sterilizer. After sterilizer starts boiling, 30 minutes vapour sterilisation shall be done.

15 Quality Control of Consumables

Chemicals

The chemicals of only highest purity of either, Analytical Reagent **(AR)** or Guaranteed Reagent **(GR),** from reputed manufacturing companies shall be used. Whenever a new chemical is to be introduced in the routine process, it is recommended to examine the post-thaw revival rates after conducting few spilt ejaculate trials (maintaining a control) with the new chemical. Assay of chemicals shall be >99%, having less impurities.

Straws

1. Straws manufactured by reputed companies are safer to use for production of quality semen. While buying straws, package volume and microbial load in straws shall be checked randomly from the consignment. In addition, some empty straws should be placed in filling and sealing machine and the machine should be run to see the sealing quality of the straws. In case of any foul smell, it should be presumed that the straws are manufactured from poor plastic which could be toxic to the spermatozoa and can even result in reduced motility on long storage.

2. The factory plug should not be loose. The factory seal should be impenetrable and the seal formed should be homogeneous and compact.

3. The straws should be intact (without cracks / dents, etc.) during and after freezing / thawing.

4. The movement of straws along the printing machine should be free and print should be clear and sharp. Print should not fade as a result of freezing and subsequent thawing.

5. The use of dark coloured straws should be avoided, as they are not transparent enough. Due to this, it is difficult to distinguish between filled / semi-filled straws.

6. Movement of the factory plug should be free.

7. Straws should be routinely checked for microbial load.

Note**:** The semen stations should avoid purchase of consumables on lowest quotation basis. For example: To produce top quality semen, it is better to use AR / GR reagents manufactured by reputed companies whose products are reliable. This is true with other consumables also.

16 Manpower Requirement for semen production

Designation	Up to 10 lakh doses	>10-25 lakh Doses	>25-50 lakh Doses	>50 lakh doses	Mega Semen Stn. 10m doses
General Manager	1	1	1	1	1
QCO/QAO	1	1	1	1	1
Vet. Officer	1	2	3	3-4	5-6
Agriculure Officer	1	1	1	1	1
Data Mgmt. Officer	--	1	1	1	1
Accts. & Adm. Officer	--	1	1	1-2	1-2
Office Assistant	1	2	3	5	6-7
Livestock Assistant	1	2	3	4	5
Agri. Assistant					
Lab Technician	1	2	3-4	5-6	8-10
Vehicle/Tractor Driver	1	2	3	4	5
Lab Attendant	2	3	3-5	7-8	10-12
Bull Attendant	1 person per 7- 8 bulls				
Agri. Labourers	15-20/100 acres depending on mechanization level				

The manpower structure suggested above is meant only for semen/fodder production. For other activities, manpower may be positioned as per the need. For dispatch of semen, facility should be

created preferably away from semen station and operated by other person/s not responsible for semen production. The GOI / Department of AH / Livestock Boards / NGO / Private agencies / Union and Federation shall review the requirement of manpower position for each semen station and finalise the staff structure for recruiting additional manpower. After recruitment, all new persons shall be trained at any of the recognized institutes. Once trained, they shall continue to work in the semen station at least for five years.

Refresher training / visit to other semen lab: technical exposure of semen station personnel working in the semen lab must be arranged compulsorily once in two to three years at reputed institutions like CFSP&TI - Hessarghatta, KLDB - Mattupatty, etc. As semen production activity is an extremely technical work, job rotation of personnel could be detrimental in maintaining the quality of semen. Therefore, personnel working in a semen station should not be transferred at least for five years. If it is inevitable, in the interest of carrying out good work, it should be essential that a proper replacement is identified at least six months in advance and is trained in semen production technology.

Annexure- 1

DEFINITIONS FOR USE IN THE HEALTH PROTOCOL

Bull	Adult male cattle or buffalo used for collection of semen. Teasers and other animals resident in the semen stations are also subjected to similar disease testing, vaccination and medications for maintaining their health status.
Bull Calf	A male cattle or buffalo which has not yet reached sexual maturity.
Known health status	Animals originating from a semen station or rearing station that is strictly complying with the guidelines mentioned in the MSP.
MSP diseases	MSP diseases are the set of diseases – the causative organism of which should not be present in the semen – or preferably in the bull. These diseases include Bovine Brucellosis, Tuberculosis (TB), Paratuberculosis (JD), Bovine Genital Campylobacteriosis, Trichomoniasis and Foot and Mouth Disease (FMD).
Quarantine station	A farm where bulls or bull calves are isolated and examined to assess the health status before shifting to the semen station or rearing station. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during quarantine.
Rearing station	A farm where bull-calves or young bulls, coming from quarantine station are reared till they attain sexual maturity and subsequently get shifted to

	semen station. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during the stay of bull calves in the rearing station to maintain their health status.
Semen station	A farm along with semen processing facilities where adult bulls are housed for semen collection and processing. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during the stay of bulls in the semen station to maintain their health status.
Unknown health status	Animals originating from village or farm where all the animals of the farm or the village have not been tested against the MSP diseases

Annexure- 2

Details of the tests to be conducted			
Disease	**Test**	**Sample**	**Tested by officers of**
Brucellosis	ELISA	Serum	CDDL/RDDL/ NDDB/PD_AD MAS
TB*	DTH- Tuberculin PPD	Intra-dermal on the bull	Semen station/ CDDL/RDDL/ NDDB
JD*	DTH- Johnin PPD	Intra-dermal on the bull	Semen station/ CDDL/RDDL/ NDDB
Trichomoniasis	Agent identification	Preputial washings / semen	CDDL/RDDL/ NDDB
Bovine Genital Campylobacteriosis	Agent identification	Preputial washings	CDDL/RDDL/ NDDB
FMD	ELISA	Serum	PD-FMD, Mukteshwar and its laboratories/ NDDB

*** TB and JD testing at Quarantine Station as well as Rearing Station shall be performed by the officers of the Semen Station. However, the testing at the Semen Station shall be done by the Officers of the CDDL/RDDL/NDDB.**

Quarantine Guidelines

Annexure- 3A

A. Quarantine of adult bulls of unknown health status		
Quarantine period	Minimum 60 days or long enough to allow at least two tests for MSP diseases to be performed during quarantine with a minimum interval of 30 days between the two tests. In case of TB and JD the interval between the two tests should not be less than 62 days.	
Shifting of bulls from the quarantine	Within 30 days from the date when the last test was performed and all bulls were found negative.	
Action on finding a positive result	Brucellosis, TB, JD, Bovine Genital Campylobacteriosis, Trichomoniasis	Cull / remove the positive bull and put all the remaining bulls under extended quarantine.
Extended quarantine	For a period of minimum 60 days or long enough to allow at least two tests for the diseases mentioned above to be performed, from the day last positive bull was culled/ removed. Perform one test within the last 30 days of the extended quarantine.	
Action on finding a positive during extended quarantine	During Quarantine, if the bulls are housed and managed • Individually - Remove only the positive bull. • In groups (not more than 3 animals in each group) – Remove all bulls in the group in which positive was detected. • Free and not in groups- Remove all the bulls.	

B. Quarantine of adult bulls of known health status	
Quarantine period	Minimum 30 days or long enough to allow at least one test for all MSP diseases
Shifting of bulls from the quarantine	Within 30 days of the last negative test
Action on finding a positive result	Same as in Annex- 3A
Extended quarantine	For a period of minimum 30 days from the day last positive bull was culled/ removed. Perform one test within the last 30 days of the extended quarantine.
Action on finding a positive during extended quarantine	Same as in Annex- 3A

C. Quarantine of adult bulls to be shifted between the farms managed by the same administration ■ For shifting between semen stations for semen production ■ From a rearing station that implements Quarantine (Annexure-3D) before allowing entry of calves for rearing	
Quarantine period	Minimum 30 days or sufficient to allow at least one test for MSP diseases
Shifting of bulls from the quarantine	Within 30 days of the last negative test
Action on finding a positive result	Same as in Annexure- 3A
Extended quarantine	For a period of 30 days from the day last positive bull was culled/ removed. Perform one test within the last 30 days of the extended quarantine.
Action on finding a positive during extended quarantine	Same as in Annexure- 3A

<table>
<tr><th colspan="3">D. Quarantine of calves above 2 months of age</th></tr>
<tr><td>Quarantine period</td><td colspan="2">Minimum 60 days or sufficient to allow at least two tests for each of the MSP diseases to be performed with a minimum interval of 30 days between the tests. In case of TB and JD the interval between the two tests should not be less than 62 days.</td></tr>
<tr><td>Shifting of calves from quarantine</td><td colspan="2">Within 30 days of negative results.</td></tr>
<tr><td rowspan="3">Action taken on finding positive calf</td><td>TB, JD</td><td>Remove the positive calf and put all the remaining calves under extended quarantine.</td></tr>
<tr><td>Bovine Genital Campylobacteriosis and Trichomoniasis</td><td>Tests conducted only on calves older than 6 months.

Remove the positive calf and put all the remaining calves under extended quarantine.</td></tr>
<tr><td>Brucellosis</td><td>Remove the positive calf irrespective of age and put all the remaining calves under extended quarantine.
OR
If the positive calf is less than 9 months old, isolate the calf till it is 9 month old and retest. Calf positive at retesting should be removed.</td></tr>
<tr><td>Extended quarantine</td><td colspan="2">For a period of minimum 60 days from the day last positive calf was removed. Perform one test within the</td></tr>
</table>

	last 30 days of the extended quarantine.
Action on finding a positive during extended quarantine	Same as in Annexure- 3A

Annexure- 4

Disease testing and management of Bovine Tuberculosis in Semen Station

Name of test	Delayed Hypersensitivity – Single Intra Dermal (SID) Test
Reagent used	Bovine tuberculin PPD
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	***Positive:*** Increase in skin thickness of 4 mm or more, or presence of clinical signs viz. exudation, necrosis, pain, and inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation. ***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. ***Inconclusive***: Increase in skin thickness more than 2mm & less than 4mm, absence of above clinical signs, 72 hours post-inoculation. Bull with inconclusive result should be immediately isolated. Only if the animal is negative during the testing in isolation, it should be brought back to the semen station.
Eligible animals	Animals above 2 months of age
Action to be	Immediate isolation and removal from herd (within

taken on Positive animal	2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since the last negative test.
Positive herd testing	Testing not before 42 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
TB free herd	Herd found negative on two consecutive tuberculin tests carried out at an interval of 6 months, the first being performed 6 months after the culling of last affected animal. If frequency of testing is more than two in a year, the testing should establish that all animals in the herd have been negative for the last 6 months beginning from 6 months after culling the last affected animal.

Annexure- 5

Disease testing and management of Paratuberculosis (JD) in Semen Station

Name of test	Delayed Hypersensitivity – Single Intra Dermal (SID) Test
Reagent used	Johnin PPD
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	***Positive:*** Increase in skin thickness of 4 mm or more, or presence of clinical signs viz. exudation, necrosis, pain, and inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation. ***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. ***Inconclusive***: Increase in skin thickness more than 2mm & less than 4mm, absence of above clinical signs, 72 hours post-inoculation. Bull with inconclusive result should be immediately isolated. Only if the animal is negative during the testing in isolation, it should be brought back to the semen station.
Eligible animals	Animals above 2 months of age
Action to be	Immediate isolation and removal from herd (within

taken on Positive animal	2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since the last negative test.
Positive herd testing	Testing not before 42 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
JD negative herd	Herd found negative on two consecutive Johnin tests carried out at an interval of 6 months, the first being performed 6 months after culling of the last affected animal. If frequency of testing is more than 2 in a year, the testing should establish that all animals in the herd have been negative for the last 6 months beginning from 6 months after culling the last affected animal.

Annexure- 6

Disease testing and management of Bovine Brucellosis in Semen Station

Name of test	Enzyme Linked Immunosorbent Assay (ELISA)
Sample required	Serum
Eligible animals	All animals. However, animals up to 9 months of age may have maternal antibodies.
Action to be taken on the positive animal	Immediate isolation and removal from herd after castration (within 2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since the last negative test.
Positive herd testing	Testing 30 to 60 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
Brucellosis free herd	Herd found negative on two consecutive annual tests. If the frequency of testing is more than one in a year, the testing should demonstrate that the herd has been negative for the last one year

Annexure- 7

Disease testing and management of Bovine Genital Campylobacteriosis (BGC) in Semen Station

Name of test	Agent –Identification
Sample required	Preputial washing/ semen
Eligible animals	Animals above 6 months of age
Positive animal	Immediate isolation and removal from herd (within 2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since the last negative test.
Positive herd testing	Minimum of 30 days after treatment/culling of last positive animal.
Negative herd testing	Annual (± 1 week) testing after last whole herd negative testing.
Bovine Genital Campylobacteriosis free herd	All animals are negative on two consecutive annual testing.

Annexure- 8

Disease testing and management of Bovine Trichomonosis in Semen Station

Name of test	Agent –Identification
Sample required	Preputial washing
Eligible animals	Animals above 6 months of age.
Action to be taken on Positive animal	Immediate isolation and removal from herd (within 2 days)
Frozen semen doses of the positive animal	Destroy frozen semen doses of the positive animal since last negative test.
Positive herd testing	Minimum of 30 days after treatment/culling of last positive animal.
Negative herd testing	Annual (± 1 week) testing after last whole herd negative testing.
Bovine Trichomonosis free herd	All animals are negative on two consecutive annual testing.

Annexure-9

Management of Foot & Mouth Disease (FMD) in Semen Station

FMD outbreak in semen station	
Immediate action to be taken	Immediate disinfection of premises and fomites. Destruction of contaminated feed & fodder by burning.
Frozen semen doses of FMD infected animal	Destroy frozen semen collected from infected animal up to one month prior to onset of outbreak.
Action to be taken on FMD infected animal	• Isolate the affected bull immediately • Affected bull is treated and rested for 90 days after recovery from clinical symptoms. • No semen collection from any infected animal during the infection and up to 3 months after last case has recovered in the farm.
Animals in the farm not affected by FMD	No semen collection from healthy bulls during the outbreak and no semen collection up to one month after the last case has recovered.
Semen Sale	If frozen semen sale is from the same campus of the SS where FMD is recorded, suspend semen sale till 30 days after the last case has recovered.
FMD outbreak in areas surrounding the SS	
Ring vaccination	Arrange immediate ring vaccination within a radius of 10 Km around the focus of infection starting from the perimeter towards the focus.
Disinfection	Disinfection of the roadsides adjacent to the farm on

	a daily basis.
Movement of fodder	Stop all fodder movement through areas of infection.
Animal movement	Stop animal movement of semen station through areas of infection.

Annexure - 10

Feeding Growing and Mature Bulls

Daily nutrient requirements of growing and mature bulls *

Body wt (kg)	gain/day (g)	DM/day (kg)	C.P. (g)	TDN (kg)	Ca (g)	P (g)	Vit. A (1000 IU)
Growing bulls							
100	750	2.8	390	1.9	11	8	4
150	750	4.3	460	2.7	15	11	6
200	750	5.7	530	3.4	18	14	8
250	750	7	610	4	21	16	10
300	750	8.2	680	4.6	23	17	13
350	750	9.3	760	5.2	24	18	15
400	700	10.2	820	5.7	25	19	17
450	600	10.4	875	5.8	26	20	19
500	400	10	885	5.6	26	20	21
550	250	10	845	5.6	25	19	23
600	100	9.8	800	5.5	24	18	26
Maintenance of mature breeding bulls							
500	-	8.3	640	4.6	20	15	21
600	-	9.6	735	5.4	22	17	26
700	-	10.9	830	6.1	25	19	30

Daily ration for Bulls

Body wt. (kg)		Calf starter (kg)	C.F. (kg)	B.P.F. (kg)	Hay (kg)	Green Fodder (kg)
Growing bulls						
100		2	-	-	0.5	6-8
150		-	-	2	0	8-10
200		-	-	2	0.5	15
300		-		2	1	ad lib.
400	a)	-		2	3	ad lib.
	b)	-	2.5	-	3	ad lib.
500	a)	-	-	2.5	2-4	ad lib.
	b)	-	3	-	2-4	ad lib.
600	a)	-	-	2.5	2-4	ad lib.
	b)	-	3	-	2-4	ad lib.
Mature breeding bulls						
500	a)	-	2.5	-	2-4	ad lib.
	b)	-	-	2	2-4	ad lib.
600		----------do----------				
700		----------do----------				

Note : 1) Mineral mixture should be supplemented as follows :

- 50 g mineral mixture for bulls up to 200 kg body weight
- 70 g mineral mixture for bulls between 200 to 350 kg body weight.

- 100 g mineral mixture for bulls above 350 kg body weight

2) Fresh water should be made available 24 hrs.

Green fodder requirement of 10 mature bulls would be approx. 125 MT per year, which can be grown in 1 hectare of land by intensive farming.

*** Source:** Ranjhan, S.K (1980). Animal nutrition & feeding practices in India,
2nd Ed., p196-212

Nutrients available in feed & fodder

	Calf starter	**C.F.**	**B.P.F.**	**Green fodder**	**Hay**
DM %	90	90	90	20-25	90
CP %	22-23	18-19	22-23	5-6	5-6
TDN %	70	62-64	65-68	55-60	55