



**PRODUCT MANUAL
FOR AZOSPIRILLUM INOCULANTS
ACCORDING TO IS 14806 : 2000**

This Product Manual shall be used as reference material by all Regional/Branch Offices & licensees to ensure coherence of practice and transparency in operation of certification under Scheme-I of Bureau of Indian Standards (Conformity Assessment) Regulations, 2018 for various products. The document may also be used by prospective applicants desirous of obtaining BIS certification licence/certificate.

| | | | |
|----|--|---|--------------------------|
| 1. | Product | : | IS 14806 : 2000 |
| | Title | : | Azospirillum Inoculants |
| | No. of Amendments | : | 02 |
| 2. | Sampling Guidelines: | | |
| a) | Raw material | : | No specific requirements |
| b) | Grouping guidelines | : | NA |
| c) | Sample Size | : | 500g |
| 3. | List of Test Equipment | : | ANNEX - A |
| 4. | Scheme of Inspection and Testing | : | ANNEX - B |
| 5. | Possible tests in a day : | | |
| | (i) Viable Cells (ii) Fineness of Carrier (iii) pH (iv) Contaminants | | |
| 6. | Scope of the Licence : | | |
| | Licence is granted to use Standard Mark as per IS 14806 : 2000 with the following scope | | |
| | Name of the product | | Azospirillum Inoculants |

ANNEX-A
TO PRODUCT MANUAL
FOR AZOSPIRILLUM INOCULANTS
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LIST OF TEST EQUIPMENTS

Major test equipment required to test as per the Indian Standard

| SI No. | Tests used in with Clause Reference | Test Equipment |
|--------|--|--|
| 1 | Viable Cells and Comtaminants Cl 4.1 & 4.3 (Annex A of IS 14806) | Analytical balance (LC-0.01g , 0.010 to 600 g), pH meter 0.01 (0 to 14), Autoclave (120 °C), BOD incubator 0.1°C (28±2 °C), Hot air oven (160 °C), Water bath/Incubator-40-45°C, Orbital shaker (reciprocal shaker) (0 to 120 RPM), Laminar airflow (0-106 Air velocity), Cyclo mixer (0 to 190 RPM), Colony counter, Pipette - 1ml, 10ml, Conical flasks – 150 ml & 250 ml, Petri dishes, screw cap tubes - 10 ml, Bunsen burner, Cotton NFB medium-Nitrogen free bromothymol blue medium (Malic acid, Potassium hydroxide, Di-potassium hydrogen phosphate, Ferrous sulphate, Manganese sulphate, Magnesium sulphate, Sodium chloride, Calcium chloride, Sodium molybdate, Bromothymol blue, Isopropyl alcohol, Agar agar), Sodium hydroxide Hydrochloric acid, Distilled water, Sterile water, Visual counting and use of MPN table (MPN table be better kept framed as an apparatus). |
| 2 | Fineness of Carrier Cl 4.2 | Analytical balance 150 to 212 μ (72 to 100 mesh) IS sieve, Lignite, Calcium carbonate, Autoclave for sterilization. |
| 3 | pH Cl 4.4 (Annex B of IS 14806) | pH meter, Analytical balance (0-200g, LC-0.01 mg), Rotary shaker (0 to 120 RPM), conical flasks –250 ml, Measuring cylinder-50ml, Funnels, Filter paper, Glass beaker, Glass rod, Standard pH buffers, Distilled water. |
| 4 | Moisture Cl 4.5 (Annex B of IS 14806) | Analytical balance (0-200g, LC-0.01 mg), Hot air oven-capable of operating at 100-105°C, Desiccator, crucible. |

| | | |
|---|---|---|
| 5 | Effective nodulation Cl 4.6 (Annex C of IS 14806) | Analytical balance (0-200g, LC-0.01 mg), Autoclave – capable of operating at 120°C, Hot air oven – capable of operating at 60°C, pH meter, Incubator- capable of operating at 30°C, Mortar & pestle, Beaker, Pipette, Seed, Earthenware/glazed pots, Soil, Coarse Sand, Scissors, screw capped bottles/ Test tubes with rubber hung, Desiccator, Pot culture house (growth rooms/cabinets), Orbital shaker (0 to 120 RPM), Plastic tube, Nitrogen Free Bromothymol Blue, Semi-Solid Malate Media, Potassium chloride, Potassium hydrogen phosphate, Calcium sulphate, Manganese sulphate, Magnesium sulphate, Copper sulphate, Zinc sulphate, Ammonium molybdate, Boric acid, Ferrous sulphate, Citric acid, Ammonium nitrate, Soft tap water/RO water, 95% alcohol, Chlorine water /0.1% mercuric chloride, Sterile water, Conc. Sulphuric acid, Calcium chloride. PLANT NUTRIENT SOLUTION: Potassium chloride, Potassium hydrogen phosphate, Calcium Sulphate, Magnesium sulphate, Trace elements solution (Copper Sulphate, Zinc Sulphate, Magnesium sulphate, Ammonium molybdate, Boric acid) Iron solution (Ferrous sulphate, Citric acid), Mortar, Distilled water. |
| 6 | Quality of broth Cl 4.7 (Annex D of IS 14806) | Laminar Air flow, NFB medium, Inoculation Loop, Incubator (28±2 °C), Microscope-100x lens, Hot air oven –capable of operating at 30 to 450 °C, Autoclave –capable of operating at 27 to 150 °C, Glass slides, pH meter, Refrigerator/freezer, Water bath, Ammonium oxalate, Gram staining kit- Crystal violet solution, Iodine solution, Ethyl alcohol, Safranin (erythrosine), Immersion oil, Test tubes containing media broth. MEDIUM: (Agar, Yeast extract, Mannitol, Potassium hydrogen phosphate, Magnesium sulphate, Sodium chloride, Congo red, Distilled water), MPN method, Bunsen burner, Filter paper. |
| 7 | Packaging Material Cl 5 | Micrometre 0-25 mm with 0.01mm LC |

The list above is indicative only and may not be considered as exhaustive.

ANNEX – B

**SCHEME OF INSPECTION AND TESTING
FOR AZOSPIRILLUM INOCULANTS
ACCORDING TO IS 14806 : 2000**

1. **LABORATORY** - A laboratory shall be maintained which shall be suitably equipped (as per the requirement given in column 2 of Table 1) and staffed, where different tests given in the specification shall be carried out in accordance with the methods given in the specification.
 - 1.1 The manufacturer shall prepare a calibration plan for the test equipment.
2. **TEST RECORDS** – The manufacturer shall maintain test records for the tests carried out to establish conformity.
3. **PACKING, STORAGE AND MARKING** – The Standard Mark as given in Schedule of the licence shall be stenciled/printed on each packet of Azospirillum Inoculants or printed on the labels applied to the packet, as the case may be, provided always that the material in each container to which this mark is thus applied conforms to every requirement of the specification.
 - 3.1 **Marking** – Marking shall be done as per clause 6 of IS 14806. In addition, the following details shall be mentioned on each pack legibly and indelibly:
 - a) BIS Licence No. CM/L__.
 - b) BIS website details i.e – “For details of BIS certification please visit www.bis.gov.in”
 - 3.2 **Packing** - Azospirillum Inoculants shall be packed in polyethylene packs, thickness which shall not be less than 75-100 micron. One sample from each consignment of the packaging material received shall be tested for its conformity to clause 5 of IS 14806.
 - 3.3 **DIRECTION FOR USE** - Direction for use of Azospirillum Inoculants, as given in Annex E of IS 14806 shall be printed briefly on the packet. A separate pamphlet may preferably be given with it.
 - 3.4 **STORAGE** - Azospirillum Inoculants shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 20°C, and not exceeding 30°C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.
3. **CONTROL UNIT** – For the purpose of this scheme, the quantity of the material blended in a blender at a time and taken from the same consignment of raw material shall constitute a control unit.

5. **LEVELS OF CONTROL** - The tests as indicated in column 1 of Table 1 and the levels of control in column 3 of Table 1, shall be carried out on the whole production of the factory which is covered by this plan and appropriate records maintained in accordance with paragraph 2 above.
 - 5.1 All the production which conforms to the Indian Standard and covered by the licence should be marked with Standard Mark.
 - 5.2 On basis of test and analysis results, the decision regarding conformity of otherwise of a control unit shall be taken as follows.
 - 5.2.1 A representative sample shall be taken from each control unit and tested for all the requirements given in Table 1 of SIT except carrier fineness.
 - 5.2.2 A sample shall be tested from each consignment of carrier material received for carrier fineness as given in Table 1 of SIT.
6. **RAW MATERIAL**- Routine analysis of each consignment of carrier material received in the factory shall be carried out and record maintained as given in Form I. Carrier should be neutralized with calcium carbonate and sterilized. Proper records shall be maintained for neutralization and sterilization.
 - 6.1 Specific mother culture be obtained from any recognized institution maintaining the mother cultures. The manufacturer may control the quality of the broth as given in Annex D of IS 14806, it should get verified at least by two institutions as mentioned in note below clause 4.7 of IS 14806.
7. **REJECTIONS** – Disposal of non-conforming product shall be done in such a way so as to ensure that there is no violation of provisions of BIS Act, 2016.

TABLE 1
LEVELS OF CONTROL

| (1) | | | | (2) | (3) | | |
|--------------|----------------------|----------------------|----------------|---|-------------------|--|--|
| Test Details | | | | Test equipment requirement R:required (or) S: Sub-contracting permitted | Levels of Control | | |
| Cl. | Requirements | Test Method Cl. Ref. | Test Method IS | | No. of Samples | Frequency | Remarks |
| 4.1 | Viable Cells | Annex A | IS 14806 | R | One | Each control unit | |
| 4.2 | Fineness of Carrier | 4.2 | -do- | R | -do- | Each consignment of the carrier material received. | See Clause 5.2.2 of SIT. |
| 4.3 | Contaminants | Annex A | -do- | R | -do- | Each control unit | |
| 4.4 | pH | Annex B | -do- | R | -do- | -do- | |
| 4.5 | Moisture | 4.2 & Annex B | -do- | R | -do- | -do- | |
| 4.6 | Effective nodulation | Annex C | -do- | R | -do- | Once in a season for each strain of Azospirillum | Record of isolation and identification of various strains of Azospirillum culture used for different crops, nodulation ability and effectiveness shall be maintained in a separate register. |

Note-1: Whether test equipment is required or sub-contracting is permitted in column 2 shall be decided by the Bureau and shall be mandatory. Sub-contracting is permitted to a laboratory recognized by the Bureau or Government laboratories empaneled by the Bureau.

Note-2: Levels of control given in column 3 are only recommendatory in nature. The manufacturer may define the control unit/batch/lot and submit his own levels of control in column 3 with proper justification for approval by BO Head.

Note-3: A Separate record shall be maintained for Quality of each batch of broth used.

FORM I
PROFORMA OF RECORD FOR CARRIER RECEIVED

| Sl. No. | Date of receipt | Source | Name of the Carrier | Sieve analysis | Colour | pH | Remarks |
|----------------|------------------------|---------------|----------------------------|-----------------------|---------------|-----------|----------------|
| | | | | | | | |