Andhra Pradesh Bio-fertilisers (Monitoring and Quality Control), Act, 2006

ANDHRA PRADESH
India

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Act 6 of 2006

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Andhra Pradesh Bio-fertilisers (Monitoring and Quality Control), Act, 2006(Act No. 6 of 2006)Last Updated 11th September, 2019[Dated 03.01.2006]An Act to provide for the maintenance of specified counts of viable cells of microorganism, volume of Biofertilizer and to refrain from the production of contraband or spurious or phyto-toxi material and description of species of micro organism in the manufacture of a particular termed Bio-firtilizer and to monitor the effectiveness of the product in the label by the manufacturer and for the matters connected there-with or incidental thereto.Be it enacted by the Legislature Assembly of the State of the Andhra Pradesh in the Fifty-sixth year of the Republic of India as follows:Chapter-I Preliminary

1. Short title, extent and commencement.

(1)This Act may be called the Andhra Pradesh Bio-fertilisers (Monitoring and Quality Control), Act, 2006.(2)It extends to the whole of the State of Andhra Pradesh.(3)It shall be deemed to have come into force with effect on and from the 21st November, 2005.

2. Definitions.

- In this Act, unless the context otherwise requires,-(1)'Bio-fertiliser' means a inert material containing microbial inclulants (living organism) as specified in Schedule I;(2)'Certificate of Source' means a certificate issued by the Government or as the case may be the Manufacturer to the Dealer indicating the source from which the Bio-fertiliser for purpose of sale is obtained:(3)'Controller' means the person appointed as Controller of Bio-fertiliser by the Government and includes any other person empowered by the Government to exercise or perform all or any of the functions as may be prescribed:(4)'Dealer' means a person carrying on the business of selling Bio-fertilisers and includes a manufacturer and an agent carrying on such business;(5)'Government' means the State

Government;(6)'Grade' means the living organism contents in Bio-fertiliser expressed in number of cells/gram of carrier material/millilitre of liquid Biofertiliser;(7)'Inspector' mean an Inspector appointed under Section 19 of the Act;(8)'Label' means any written, printed or graphic matter on the package and any other covering in which the package is placed or packed and includes any written, printed, or graphic matter accompanying the Biofertiliser;(9)'Manufacturer' means a person who produces Bio-fertilisers and expression manufacturer with its grammatical variations shall be construed accordingly;.(10)'Non-standard Bio-fertiliser' means,-(i)if its label contain any statement, design or graphic statement/ representation thereto which is false or misleading in any material particular, of if its package is otherwise deceptive in respect of its contain; or(ii)if it is an imitation of, or is sold under the name of another Biofertiliser; or(iii)if any word, statement or other information required by or under this Act to appear on the label is not displayed thereon in such conspicuous manner as the other words, statements or graphic matter have been displayed on the label and in such terms as to render it likely to be read and understood by any ordinary individual under customary conditions of purchase and use; or(iv)if it is not packed and labelled as required by or under this Act; or(v)if it is not registered in the manner required by or under this Act; or(vi)if the lable contains any reference to registration other than the registration number; or(vii)if the bio-fertiliser is not in conformity with the prescribed standards as specified in Section 2 (16) of this Act and packed with any substance which is not included in the registration;(11)'Notification' means a notification published in the Andhra Pradesh Gazette and the word 'notified' shall be construed accordingly;(12)'Officer for sale' includes a reference to intimation by a person and a person who proposed for sale, exhibit the price list, indicating the prices of Biofertiliser;(13)'Package' means a box, bottle, casket, tin, barrel, case resceptacle, sack, bag wrapper or other thing in which a bio-fertilliser is placed or packed;(14)'premises' means any land, shop, stall or place where any bio-fertiliser is manufactured or stored or sold or used and includes and vehicle carrying bio-fertilisers;(15)'prescribed' means prescribed by rules made under this Act;(16)'Prescribed standard' means prescribed under this Act;(17)'Registering Authority' means a registering authority appointed under Section 18;(18)'Schedule' means the Schedule appended to this Act;(19)'State' means the State of Andhra Pradesh.Chapter-II Price Control

3. Fixation of prices of Bio-Fertilisers.

(1)The Government may be notifications regulate equitable distribution and also the rates of Bio-fertilisers in the manner as may be prescribed.(2)The Government may having regard the local conditions of any area, the period of storage of Bio-fertilisers, and other relevant circumstances, fix different prices or rates for Biofertilisers having different periods of storage or for different classes of consumers.(3)No dealer or manufacturer shall sell or offer for sale any bio-fertiliser at a price exceeding the maximum price or rate fixed under this Act or printed on the container.

4. Display of Stock Position and Price List.

- Every dealer, who makes or offers to make a sale of any Bio-fertlisier, shall prominently display in his place of business;-(i)The quantities of opening stock of different Bo-fertilisers held by him on each day.Explanation. - The actual stocks at any point of time during the day may be different from that of the displayed opening stock to the extent of sale and receipt of such Bio-fertilisers up to the

time of inspection during the day.(ii)A list of prices or rates of such Bio-fertilisers fixed under Section 3 or the Maximum Retail Price printed on the container by the manufacturer.

5. Issue of cash or credit memorandum.

- Every dealer shall issue a cash or credit memorandum to a purchaser of bio-fertilisers in the manner prescribed.

6. Control on distribution of Bio-fertilisers by manufacturers.

- The Government may be notification direct any manufacturer, to secure equitable distribution and availability of bio-fertiliser to the farmers in time, to sell the bio-fertilisers produced by any person in such quantities in such areas of the State and within such periods as maybe specified in the notification.

Chapter III
Registration of Manufacturers / Dealers

7. Dealers to be registered.

- No person including manufacturer/ dealer shall offer for sale or carry on business for selling Bio-fertilisers at any place except under and in accordance with terms and conditions of its certificate of registration granted to any person as prescribed, under Section 8 of this Act:Provided that the Government may if it considers necessary or expedient by notification exempt from the provisions of this section any person selling Biofertilisers to the farmers in such areas and subject to such conditions as may be specified in that notification.

8. Application for Registration.

(1)Every person desiring to obtain a Certificate of Registration under this Act for manufacturing bio-fertilisers by a manufacturer shall make an application for registration to the Registering Authority or the Controller in the manner prescribed.(2)Every person desiring to obtain a Certificate of Registration under this Act for selling Bio-fertilisers by a Dealer shall make an application for registration to the Registering Authority together with the fee in the manner prescribed.Provided that where the applicant is a Government or a manufacturer, it shall not be necessary to enclose a certificate of source along with the application: Provided further that where Biofertiliser, are obtained for sale from different sources, a certificate of source from each source shall be furnished in the manner prescribed:Provided also that a dealer, except a manufacturer shall not issue a certificate of source to authorize another dealer for dealing in Bio-fertiliser.

9. Grant of refusal of Certificate of Registration.

- The Registering Authority or as the case may be the Controller shall grant a Certificate of Registration within 30 (thirty) days of the receipt of the application to any person in the manner prescribed:Provided that no Certificate of Registration shall be granted to a person,-(i)if his previous Certificate of Registration is under suspension: or(ii)if his previous Certificate of Registration has been cancelled within a period of one year immediately preceding the date of making the application; or(iii)If he has been convicted of any offence under his Act or any order made thereunder within three years immediately preceding the date of making the application; or(iv)if he fails to enclose with the application, a certificate of source; or(v)if the application is incomplete in any respect.

10. Period of validity of Certificate of Registration.

- Every Certificate of Registration granted Section 9 or as the case may be renewed under Section 11, unless suspended or cancelled shall be valid for a maximum period of 3 (three) years from the date of issue.

11. Renewal of certificate of Registration.

(1)Every holder of a Certificate of registration desiring to renew the Certificate of registration granted under Section 9 shall, before the date of expiry of such certificate of registration make an application for renewal to the Registering Authority or as the case maybe, to the Controller as may be prescribed in duplicate, together with the fee prescribed under Section 30 of the Act for such renewal and a certificate of source as required under Section 8 of the Act.(2)On receipt of such application together with such fee and certificate of source, the Registering Authority, or as the case may be, the Controller may renew the certificate of registration as may be prescribed:Provided that a Certificate of registration shall not be renewed if the holder of the certificate of registration did not sell any bio-fertiliser during the period of one year immediately preceding the date of expiry of the period of validity of the Certificate of registration sought to be renewed.(3)If any application for renewal is not made before the expiry of the period of validity of the Certificate of registration but is made with in one month from the date of such expiry, the Certificate of registration may be renewed on payment of such additional fee as may be prescribed by the Government, or as the case may be the Controller, in addition to the fee for renewal of the certificate of registration.(4)Where the application for renewal is made within the time specified in sub section (1) or sub section (3) the application shall be deemed to have held a valid certificate of registration, until such date as the Registering Authority or as the case may be the Controller passes orders on the application for renewal.(5)If an application for renewal of a certificate of registration is not made with in one month from the date of expiry of the period of validity of the certificate of registration, the certificate or registration shall be deemed to have lapsed on the date on which its validity expired and any business carried on or after that date shall be deemed to have been carried on in contravention of Section 7 of the Act.

Chapter IV
Restriction on Manufacture, Sale etc. of Bio-fertilisers

12. Restriction on Manufacture sale and distribution of Bio-fertilisers.

(1)No person shall himself or by any other person on his behalf,-(i)manufacture for sale, sell, offer for sale, stock or exhibit for sale or distribute any Bio-fertiliser which is not prescribed standard:(ii)manufacture for sale, sell, offer for sale, stock or exhibit for sale, or distribute any Bio-fertiliser, which is not of prescribed standard and which does not conform to the particulars specified in the certificate of manufacture granted to him under this Act in respect of Bio-fertilisers;(iii)sell, offer for sale, stock or exhibit for sale or distribute.-(a)any Bio-fertiliser, the container whereof is not packed and marked in the manner laid down in this Act;(b)any Bio-fertiliser which is an imitation or substitute for another Bio-fertiliser under the name of which it is sold;(c)any Bio-Fertiliser which is adulterated.Explanation. - A Bio-Fertiliser shall be deemed to be adulterated, if it contains any substance addition of which is likely to eliminate or decrease the living organisms or make the fertilisers not conforming to the prescribed standard;(d)any Bio-fertiliser, the label or container whereof bears the name of any individual, firm or company purporting to be manufacturer of the Bio-fertiliser, which individual, firm or company is fictitious or does not exist;(e)any Bio-fertiliser, the label or container whereof or any thing accompanying therewith bears any statement which makes a false claim for the Bio-fertiliser or which is false or misleading in any material particular;(f)any substance purported to be a Bio-fertiliser which substance is not, infact, a Bio-fertiliser; or(g)any bio-fertiliser which is not properly labelled by exhibiting the minimum guaranteed count of living organisms.(2)Any manufacturer who intends to manufacture for sale/offer for sale/ stock, or exhibit for sale or distribute any bio-fertiliser other than those specified in the Schedule-I shall make an application to the prescribed authority and the same shall be scrutinized by the Technical Committee appointed for this purpose by the Government and be recommended to the Government for final orders by notification by the Government in the manner prescribed.

13. Manufacturers to comply with certain requirements for Laboratory facility.

- Every manufacturer shall is order to ensure the quality of their product possess minimum lab facility as may be specified form time to time by the Controller.

14. Bulk sale of Bio-fertilisers.

- Notwithstanding any thing contained in this Act,-(i)a dealer shall not retain any container of any variety of bio-fertiliser in an open and unsealed condition for the purpose of sale;(ii)the Government may by notification authorize a manufacturer to sell any Bio-Fertiliser manufactured by him in bulk also direct to the farmers for such period as may be specified in that notification:Provided that certificate indicating the minimum living cells be issued by the manufacturer to each farmer at the time of such sale.

15. Disposal of non-standard Bio-fertilisers.

(1)Not withstanding any thing contained in this Act, no person shall sell or offer for sale, stock, exhibit for sale or distribute, any bio-fertiliser which does not conform to the prescribed standards unless the Bio-fertiliser is reprocessed by the manufacturer under the supervision of enforcing authorities, in the manner prescribed.

16. Manufacturers to appoint officers responsible for compliance of the Act.

- Every manufacturing organization shall in consultation with the Government, appoint an officer in that organisation who shall be responsible for compliance with the provisions of this Act.

17. Restriction of sale/use of Biofertilisers.

- No person shall, except with prior permission of the Government, and subject to such terms and conditions as may be imposed by the Government, sell or use Bio-Fertilizers for purpose other than fertilisation of soils and increasing productivity of crops.Chapter-V Enforcement Authorities

18.

(1)The Government may by notification appoint an officer as the Controller, for the purposes of this Act.(2)Appointment of Controller and Registering Authority. - The Government may by notification appoint such number of persons as it thinks necessary, to be the registering authorities for the purposes of this Act and may in any such notification define the limits of the local area within which each such registering authority shall exercise his jurisdiction.

19. Appointment of Inspectors.

- The Government may be notification appoint such number of persons, as it thinks necessary to be Inspectors of Bio-Fertilisers for the purpose of this Act and may in such notification define the limits of local area within each such inspector shall exercise his jurisdiction.

20. Qualification for appointment of Inspectors.

- No person shall be eligible for appointment as Bio-fertiliser Inspector, under this Act unless he possesses the qualification namely Graduation in Agriculture from a recognized University and working in the Department of Agriculture, in the State.

21. Powers of Inspectors.

(1)An Inspector may with a view to securing compliance with this Act,-(i)require any manufacturer or a dealer to give any information in his possession, with respect to the manufacture, storage and disposal of any Bio-fertiliser manufactured or in any manner handled by him;(ii)draw samples of

any Bio fertilizer in accordance with the procedure for drawal of samples laid down in Schedule II.Provided that the Inspector shall prepare the sampling details in duplicate as may be prescribed and hand over one copy of the same to the dealer or his representative from whom the sample has been drawn;(iii)enter upon and search any premises where any Bio-fertiliser is manufactured or stored or exhibited for sale, if he has reason to believe that any Bio-fertiliser has been or is being manufactured, sold, offered for sale, stored, exhibited for sale or distributed contrary to the provisions of this Act;(iv)seize or detain any Bio-fertiliser in respect of which he has reason to believe that a contravention of this Act has been or is being or is about to be committed;(v)seize any books of accounts or documents relating to manufacture, storage or sale of Bio-fertiliser etc in respect of which he has reason to believe that any contravention of this Act has been or is being or is about to be committed:Provided that the Inspector shall give a receipt for such Bio-fertilisers or books of accounts or documents so seized to the person from whom the same has been seized:Provided further that the books of accounts or documents so seized shall be returned to the person from whom they were seized after copies thereof or extracts therefrom as certified by such person, have been taken:Provided also that the Inspector shall give the stop sale notice in writing to the person whose stocks have been detained and initiate appropriate action as per the provisions of this Act or the rules made thereunder within a period of twenty one days. If no action has been initiated by the Inspector within the said period of twenty one days from the date of issue of the said notice, the notice of stop sale shall be deemed to have been revoked.(2)Where any Bio-fertiliser is seized by any Inspector under this section he shall forthwith report the fact of seizure to the respective Magistrate and seek further orders.

Chapter VI
Analysis of Samples

22. Analysis of samples.

- Bio-fertiliser sample drawn by an Inspector shall be analyzed in accordance with the standards laid down in Schedule -I as per the specification of Bureau of Indian Standards.

23. Qualification for appointment of Bio-fertiliser Analyst in the Bio-fertiliser Control Laboratories.

- No person shall be eligible for appointment as Bio-fertiliser analyst for analysis of Biofertiliser samples in the laboratories as may be notified for the purposes of this Act, unless he possesses the qualification namely Graduation in Agriculture from a recognized University with training in Bio-fertilisers quality control and analysis at National Centre for Organic Farming, Ghazibad, or its regional centers:Provided that the Bio-fertiliser analysts appointed before the commencement of this Act, who do not possess the requisite training, shall undergo the prescribed training with a period of one year National Centre for Organic Farming, Ghazibad, or its regional centres.

24. Time limit for analysis and communication of results.

(1)Where a sample of Bio-fertiliser has been drawn, the same shall be dispatched along with memorandum in the manner pi ascribed to such laboratory as may be notified for analysis within a period of 7 (seven) days from the date of its drawal.(2)The Laboratory shall analyse the sample and forward the analysis report in the form as may be prescribed within 30 (thirty) days from the date of receipt of the sample in the laboratory to the authorities specified in the said memorandum.(3)The authority to whom the analysis report is sent under sub-section (2) shall communicate the result of analysis to the dealer/manufacturer from whom the sample was drawn within 15 (fifteen) days from the date of receipt of analysis report.(4)The report of the Laboratory is final for initiating any criminal proceedings against the dealer/manufacturer.

Chapter VII
Miscellaneous

25. Suspension cancellation of Registration Certificate.

(1)A registering authority or, as the case maybe, the Controller may, after giving the holder of a certificate of registration, or a certificate of manufacture or any other certificate granted under this Act, an opportunity of being heard, suspend or cancel such certificate on any of the following grounds namely: -(i)that such certificate has been obtained by fraud or misrepresentation as to material particulars:(ii)that any of the provisions of this Act or any of the terms and conditions of such certificate has been contravened or not fulfilled.Provided that while canceling the certificate the holder thereof may be allowed a period of 30 (thirty) days to dispose of the balance stock of bio-fertilisers if any held by him:Provided further that the stock of Bio-fertiliser lying with the holder after the expiry of the said 30 (thirty) days period shall be confiscated:(2)Where the contravention alleged to have been committed by a person is such as should, on being proved, justify canceling of the certificate of registration or, as the case may be certificate of manufacture or any other certificate granted under this Act to such person the registering authority or as the case may be, the Controller may without any notice, suspend such certificate as an interim measure:Provided that the registering authority, or as the case may be Controller shall immediately furnish to the affected person/persons details and the nature of contravention alleged to have been committed by such person/persons and, after giving the person/persons an opportunity of being heard, pass final orders either revoking the order of suspension or canceling such certificate within fifteen days from the date of issue of the order of suspension:Provided further that where no final order is passed within the period as specified above, the order of interim suspension shall be deemed to have been revoked without prejudice, however to any further action which the Registering authority or, as the case may be the Controller may take against the holder of the certificate of registration under sub-section (1).(3)Whenever a certificate is suspended or cancelled under this section, the registering authority or as the case may be, the controller shall record a brief statement of reasons for such suspension or, as the case may be cancellation and furnish a copy thereof to the person whose certificate has been suspended or cancelled.(4)Whenever a person is alleged to have committed the contravention, the registering authority shall, within a period of fifteen days from the

date of issue of such order of suspension or cancellation furnish to the Controller also, besides sending the same to the person whose certificate has been suspended or cancelled, a detailed report about the nature of contravention committed and a brief statement of the seasons for such suspension or, as the case may be cancellation:Provided that the Controller, shall in case of the order of suspension passed by the registering authority, on receipt of the detailed report and after giving the person an opportunity of being heard, pass final order either revoking the order of suspension or cancelling the certificate of registration, within fifteen days from the date of receipt of the detailed report from the registering authority, failing which the order of interim suspension passed by the registering authority shall be deemed to have been revoked, without prejudice however to any further action which the Controller may take against the holder of certificate of registration under subsection (1). Provided further that the order of cancellation passed by the registering authority shall remain effective as if it has been passed by the Controller till such time the Controller, on receipt of the detailed report from the registering authority and if deemed necessary after giving the person a fresh opportunity of being heard, pass the final order either revoking or confirming the order of cancellation.

26. Appeal.

- Any person aggrieved by any of the following orders or action of registering Authority or as the case may be the Controller may appeal to such authority as may be prescribed by the Government,-(i)refusing to grant, amend or renew the certificate of registration for sale of Bio-fertilisers.(ii)refusing to grant a certificate of manufacture for production of Bio-fertilisers.(iii)suspending or cancelling a certificate of registration of a manufacturer/ dealer.(iv)Non issuance of certificate of Registration to any person within a period of 60 (sixty) days from the date of receipt of such order or as the case may be from the date of expiry of such stipulated period, and the decision of such authority shall be final.

27. Grant of duplicate copies of certificate of Registration etc.

- Where a certificate of registration or a certificate of manufacture or any other certificate granted or as the case may, be renewed under this Act is lost, tom or spoiled or defaced as the case may be, the registering authority or as the case may be, the Controller may on an application made in this behalf together with the fee prescribed for the purpose under Section 30 of the Act grant a duplicate copy of such certificate.

28. Amendment of Certificate of Registration.

- The registering authority or as the case may be the Controller may amend the certificate of Registration on an application made in this behalf together with the fee as prescribed under Section 30 of the Act.

29. Maintenance of Records and submission of returns etc.

(1)The Controller may be an order made in writing direct the dealer/ manufacturer, -(i)to maintain such books of accounts, records etc, relating to their business in form as may be prescribed, and(ii)to submit to such authority, returns and statements in such form and containing such information relating to their business and within such time as may be specified in this Act.(2)Where a person holds certificate of registration for sale of Bio fertilisers, he shall maintain separate books of Account for the sales made by him.(3)Where the Government or a manufacturer holds a valid certificate of registration for sale of Bio-fertilisers, they shall maintain separate books of accounts for the sales made by them.

30. Fees.

(1)The fees payable for grant, amendment or renewal of certificate of registration, or certificate of manufacture, or grant of duplicate of such certificates or renewal thereof under this Act shall be such as the Government may from time to time fix for different purposes.(2)The authority to whom and the manner in which the fee fixed under subsection (1) shall be such as may be notified by the Government.(3)Any fee paid under sub-section (1) shall not be refundable unless the grant or renewal of any certificate of registration or certificate of manufacture to grant of duplicate copy of such certificate or renewal under this Act has been refused.

31. Offences and penalities.

(1)Whoever, -(i)manufactures, sells, stocks or exhibits for sale or distributes any Biofertiliser deemed to be nonstandard under Section 2 (10); or(ii)manufactures, sells, stocks or exhibits for sale or distributes a Bio fertiliser without a certificate of Registration; or(iii)manufactures, sells or distributes a Bio-fertiliser in contravention of Section 12; or(iv)obstructs a Bio-fertiliser inspector in the exercise of his powers of charge of this Act,-shall be punishable,-(a)for the first offence, with imprisonment for a term which may extend to one year or with fine which may extend to rupees fifty thousand, or with both;(b)for the second and subsequent offence with imprisonment of a term which may extend to two years or with fine which may extend to rupees one lakh, or with both.(2)Whoever contravenes any other provisions of this Act or any conditions of Certificate of registration granted thereunder shall be punishable;-(a)for the first offence with imprisonment which may extend to six months or with fine which may extend to five thousand rupees or with both;(b)for the second subsequent offence with imprisonment for a term which may extend to two years, or with fine which may effected to twenty thousand or with both.(3)If any person convicted by the under this Act committees a like offence afterwards, it shall be lawful for the Court before which the second or subsequent conviction takes place to cause the offenders name and place of residence, the offence and penalty imposed to be published in such newspapers or in such other manner as the court may direct.

32. Protection of action taken in good faith.

- No suit, prosecution or other legal proceedings shall lie against the Government or any officer, authority or person empowered to exercise the powers and perform the functions by or under this Act for anything which is done in good faith or intended to be done under this Act or the rules or orders made thereunder.

33. Power to remove difficulties.

- If any doubt or difficulty arises in giving effect to the provisions of this Act, the Government may, by order make such provisions or give such directions not inconsistent with the provisions of this Act as may appear to it to be necessary or expedient for removal of doubt or difficulty:

34. Power to make Rules.

(1)The Government may, by notification, make rules for carrying out all or any of the purpose of this Act.(2)Every rule made under this Act shall immediately after it is made, be laid before the Legislative Assembly of the State, if it is in session and if it is not in session, in the session immediately following for a total period of fourteen days which may be comprised in one session or in two successive sessions, and if, before the expiration of the session in which it is so laid or the session immediately following the Legislative Assembly agrees in making any modification in the rule or in the annulment of the rule, the rule shall, from the date on which the modification or annulment is notified, have effect only in such modified from or shall stand annulled as the case may be, so however, that any such modification or annulment shall be without prejudice to the validity of anything previously done under that rule.

35. Repeal of Ordinance 23 of 2005.

- The Andhra Pradesh Bio-Fertilisers (Monitoring and Quality Control) Ordinance, 2005 is hereby repealed.

I

(See Section 2(1))Part-A Specifications of Bio Fertilisers

1. Nitrogen Fixing Bio-Fertilisers: -

A. Rhizobium Inoculants (RI) (IS 8268 - 2001)(i)RI shall be carried based the colour depending on the colour of the carrier(ii)RI shall contain a minimum of 10(7) viable Rhizobium cells/g of the carrier on dry - mass basis till 6 months expiry period from the date of manufacture. The number shall be counted by the plate count method as given in 4.2 and 4.3 of 1A of Part B of Schedule-II.(iii)RI shall have a maximum of six months expiry period from the date of manufacture.(iv)RI shall have no contamination with other micro-organisms at 10(5) dilution when

counted as given in 4.3 of 1 A of Part B of Schedule- II.(v)The pH of RI shall be between 6.5 and 7.5 when tested as given in Annexure-A.(vi)RI shall show effectiveness nodulation on all those species and / or cultivars on the packet before the expiry date when tested. If good effectiveness pink nodulation is obtainable in the inoculated species together with total absence or sometimes presence of stray nodules in the controls, it should be concluded that RI contains effective Rhizobium the total dry mass of inoculated plants shall be significantly higher than that of the un-inoculated controls and at least 50 percent more than the controls.(vii)The carrier material such as peat, lignite, peatsoil, humus favouring growth to be neutralized with calcium carbonate and sterilized shall be in the form of a powder capable of passing through 150 to 212 micron (72 to 100 mesh) IS sieve.(viii)Specified mother culture be obtained from any recognized institution maintaining the mother cultures. The manufacturer may control the quality of the broth, it should get verified at least by two institutions as mentioned below:Note. - At present National Bio-fertiliser Development center and (NBDC), Ghaziabad and its Six Regional Centres located at Bangalore, Bhubaneshwar, Imphal, Hissar, Jabalpur and Nagpur, Indian Agricultural Research Institute (IARI), New Delhi, Tamil Nadu Agricultural University (TNAU) Coimbatore, University of Agricultural Science, Bangalore are sources for supplying the mother culture.(ix)The RI carrier shall be in the form of fribal (moist) with 30-40 percent (m/ m) moisture content when tested as given in Annex-B.(B)Azotobacter Chroococcum Inoculants (AI) (IS 9138 -2002)(i)AI shall be carrier- based, the colour depending on the colour of the carrier.(ii)AI shall contain a minimum of 10(7) viable Azotobacter cell/g of the carrier on dry - mass basis till 6 months expiry period from the date of manufacture. The number shall be counted by the plate count method as given in 4.2 of 1 A of Para B of Schedule II and Azotobactor Chroococcum colonies are gummy, raised with or without striations, viscous and often sticky. The pigmentation varies from very light brown to black. Count the colony number and observe the cyst formation as given Part B - Schedule II (4.2) and calculate number per gram of the carrier material.(iii)AI shall have a maximum of six months expiry period from the date of manufacture.(iv)AI shall have no contamination with other microorganisms at 10(5) dilution.(v)The pH of AI shall be between 6.5 and 7.5 when tested as given in -A.(vi)The carrier material such as peat, lignite, peatsoil, humus favouring growth to be neutralized with calcium carbonate and sterilized shall be in the form of a powder capable of passing through 150 to 212 micron (72 to 100 mesh) IS sieve.(vii)Specified mother culture be obtained from any recognized institution maintaining the mother cultures. The manufacturer may control the quality of the broth, it should get verified atleast by two institutions as mentioned in VIII of 1 of Part A of Schedule -I.(viii)The AI carrier shall be in the form of fribal (moist) with 30-40 percent (m/ m) moisture content when tested as given in Annexure-BC. Azospirillum Inoculants (ASI) (IS 14806-2000)(i)ASI shall contain 10(7) viable Azospirillum cells / g of the carrier material on dry mass basis.(ii)ASI shall no contamination with other microorganisms at 10(5) dilution. ASI contamination in semi solid medium should be checked by semi dilution and spread plate method with solid complete medium.(iii)The pH of ASI shall be 6.5 to 7.5 when tested as given in Annexure-A(iv)The ASI carrier shall be in the form of fribal (moist) with 30-40 percent moisture. When tested as given in Annexure-B(v)ASI shall show effective root development on all cultivars/crops against which the Inoculant is intended to be used.(vi)Specified mother culture be obtained from any institution maintaining the mother cultures. The manufacturer may control the quality of the both, it should get verified atleast by two institutions as mentioned in VIII of I of past A of schedule-I.

2. Phosphate Solubilising Bacterial Inoculant (PSBI) (IS 14807-2000)

(i)PSIB shall contain 10(7) available phosphate solubilising bacterial cells / g of the carrier material on dry mass basis.(ii)PSBI shall be carrier based colour depending on the colour of the carrier. Carrier material such as peat, lignite charcoal may be used. It shall be neutralized with calcium carbonate and then sterlised. When tested it shall pass through 100 micron sieve.(iii)PSBI shall have no contamination with other microorganisms at 10(5) dilution.(iv)The pH of PSBI shall be 6.5 to 7.5.(v)The PSBI carrier shall be in the form of fribal (moist) with 30-40 percent (m/m) moisture content when tested as given in Annexure-B.(vi)PSBI shall have phosphate solubilising capacity in the range of minimum 30 percent in terms of zone formation minimum 30 percent in terms of zone formation minimum 10 mm solubilisation zone in a prescribed solid having at least 3 mm thickness. When tested by the method prescribed in Annexure-C.(vii)Specified mother culture be obtained from any recognized institution maintaining the mother culture. The manufacturer may control the quality of broth, it should get verified at least by two institutions mentioned in VIII of I of Part A of schedule-I.

I

Part B – Packing Marking and Storage

1. Rhizobium:

1. Packing. - RI shall be packed material of low density polyethylene / polypropylene bags thickness of which shall be 75-100 micron minimum.

2. Marking. - Each packet shall be marked legibly to give the following information:

(i)Name of the product, specifically as Rhizobium inoculant(ii)Leguminous crop for which intended(iii)Name and address of the manufacturer(iv)Type of the carrier;(v)Batch or code number(vi)Date of manufacture(vii)Date of expiry (agreed between the manufacturer and the purchaser subject to minimum 6 months from the date of manufacture)(viii)Net quantity and the area meant for(ix)Storage instructions worded as under: STORE IN COOL PLACE AWAY FROM DIRECT SUN AND HEAT'(x)Any other information.

3. Item (ii), (vi) and (vii) shall be printed on a colour ink background

4. Directors for the use of RI shall be printed brief on the packet as given in - Annexure D. A separate pamphlet may preferably be given with it.

5. The product may also be marked with the standard mark

6. The use of the standard mark in governed by the provisions of the Bureau of Indian Standards Act 1986 and the rules and regulations made there under. The details of conditions under which the license for the use of the standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

7. RI shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 15 C to 30 C. It shall also be the duty of the manufacture to instruct the retailers and. in turn the users about the precautions to be taken during storage.

2. Azotobactor:

1. Packing. - AI shall be packed in packaging material of low density polythene / polypropylene bags thickness of which shall be 75-100 micron minimum.

2. Marking. - Each packet shall be marked legibly to give the following information.

(a)Name of the product, specifically as Azotobactor inoculant;(b)Non-leguminous crop for which intended(c)Name and address of the manufacturer(d)Type of the carrier(e)Batch of code number(f)Date of expiry (agreed between the manufacturer and the purchaser subject to minimum 6 months from the date of manufacture)(g)Net quantity and rate of application.(h)Storage instructions worded as under Store in Cool Place Away From Direct Sun and Heat(i)Any other information.

3. Direction for the use of AI shall be printed briefly on the packet as given in Annexure-D. A separate pamphlet may preferably be given with it.

4. The product may also be marked with the standard mark.

5. The use of the standard mark is governed by the provisions of the Bureau of Indian Standards Act, 1986 and the rules and regulations made there under. The details of conditions under which the license for the use of standard mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6. Al shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 15°C to 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. Azospirillium

1. Packing. - ASl shall be packed in polyethylene packs, thickness which shall not be less than 75-100 micron.

2. Marking. - Each polyethylene packs shall be marked legibility and indelibly with the following information.

(a)Name of the product, specially as Azospirillium inoculant.(b)Name and address of the manufacturer(c)Crop (S) for which intended(d)Type of the carrier used.(e)Batch number(f)Date of manufacture(g)Expiry date which shall not be less than months from the date of manufacture(h)Net mass in kg and area meant for;(i)Storage instructions worded as under: STORE IN COOL PLACE AWAY FROM DIRECT SUNLIGHT AND HEAT.(j)Any other information required under the Standards of Weights and Measures (packaged commodities) Rules 1977

3. Direction for use of ASl shall be printed briefly on the packets as given in Annexure -D of the standard. A separate pamphlet may preferable by given with it.

4. The product may also be marked with the Standard Mark.

5. The use of the Standard Mark is governed by the provisions of Bureau of Indian Standards Act, 1986 and rules and regulations made there under. The details of conditions under which the license for the use of standard mark may be granted to manufacturer or producers may be obtained from the Bureau of Indian Standards.

6. ASl 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.

4. Phosphates Solublising Bacterial Inoculant (PSBI)

1. Packing. - PSBI shall be packed in polyethylene packs, thickness of which shall not be less than 100 micron.

2. Marking. - Each polyethylene packs shall be marked legibly and indelibly with the following information.

(a)Name of the product, specially as Phosphate solubilising bacterial inoculant;(b)Name and address of the manufacturer.(c)Crop (S) for which intended(d)Type of the carrier used.(e)Batch number(f)Date of manufacture(g)Expiry date which shall not be less than 6 months from the date of manufacture(h)Net mass in kg and area meant for;(i)Storage instructions worded as under. - Store In Cool Place Away From Direct Sunlight and Heat.

3. Direction for use of PSBI shall be printed briefly on the packets as given in Annexure-D of the standard. A separate pamphlet may preferably be given with it.

4. The product may also be marked with the Standard Mark.

5. The use of the Standard Mark is governed by the provisions of Bureau of Indian Standards Act, 1986 and rules and regulations made there under. The details of conditions under which the license for the use of standard mark may be granted to manufacturer or producer may be obtained from the Bureau of Indian Standards.

6. PSBI shall be stored by the manufacturer in 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.

II

Part-A (See Section 21)Procedure for Sampling of Biofertilisers

1. General Requirements. - 1.0. In drawing, preparing and handling the samples, following precautions and direction shall be observed.

1.1Sampling shall be carried out by a trained and experienced person as it is essential that the sample should be representative of the lot to be examined.1.2Since the samples are also required for

micro biological analysis, utmost care is necessary to avoid extraneous contamination while drawing and handling the samples and to preserve them in their original conditions till they are ready for examination in the laboratory.1.2.1. No preservation or bacteriicidal/ fungicidal agent shall be added to samples required for micro-biological analysis.1.3Simples in their original up opened packets should be drawn and sent to the laboratory. This will prevent possible contamination of the samples during handling and also help in revealing the true conditions of the material.1.4Intact packets shall be drawn from a protected place not exposed to dampness, air, light, dust or soot and transferred to clean containers.1.5The sampling appliances and sample containers shall be sterile.1.6All precautions shall be taken to protect the sample the material being sampled, the sampling instruments and the sample container against adventitious contamination at the time of drawing the sample, opening containers and transferring the samples.

2. Sample Equipment. - 2.1. A suitable scoop made of stainless steel may be used for drawing samples.

2.2The sampling equipment shall be perfectly clean and sterile. It shall be properly sterlised by heating in a hot air oven at 160°C for not less than 2 h or by autoclaving for not less than 20 min at 120°C and held in suitable containers to prevent recontamination.

3. Scale of Sampling. - 3.1. Lot

All units (containers in a single consignment of type of material belonging to the same batch of manufacture) shall constitute a lot. If a consignment consists of different batches of the manufacture the containers of the same batch shall be separated and shall constitute a separate lot.3.2Batch. - An inoculant prepared from a batch fermentor or a group of flasks (containers) constitute a batch.3.3For ascertaining conformity of the material to the requirements of the specification, samples shall be tested from each lot separately.3.4The number of packets to be selected from a lot shall depend on the size of the lot and these packets shall be selected at random and in order to ensure the randomness of selection.

4. Drawl of samples. - Three(3) samples should be drawn separately from each lot as per 1.3 of part A of schedule-11. One sample should be sent for analysis, one has to be handed over to the dealer under acknowledgement, and one will be treated as referee sample.

4.2The samples should be put in a cloth bag which may be sealed as specified in From K along with other details like sample no. / code no. which enables its identification.

II

Part-B (See Section 22)

1. A. Method of Analysis of Rhizobium Bio-Fertilisers

1. Apparatus. - 1.1. Pipettes Graduated 1 ml and 10 ml

1.2Dilution Bottles or Flasks1.3Petri Dishes Clear, Uniform, flat-bottomed.1.4Hot - Air Oven Capable of giving uniform and adequate temperature equipped with a thermometer, calibrated to read up to 250 C and with vents suitably located to assure prompt and uniform heating.1.5Autoclave1.6Incubator1.7Hand Tally or Mechanical counting Device1.8pH meter

2. Reagents. - 2.1. Congo Red and one percent aqueous solution

2.2MediumUse a plating medium of the following composition

Agar	20 g
Yeast Extract	1 g
Mannitol	10 g
Potassium hydrogen phosphate (K2HP04)	0.5 g
Magnesium sulphate (MgS04 7H20)	0.2 g
Sodium Chloride (NaCL)	0.1 g
Congo red	2.5 ml
Distilled water	1000 ml
PH	7.0

2.3Sterlising and preparation procedure for plates:

2.

3.

l. Sterlise the sampling and plating equipment with diy heat in a hot air oven at not less than 160°C for not less than 2 hours.2.3.2. Sterlise the media by autoclaving at 120°C for 20 min. To permit passage of steam into and from closed containers when autoclaved, keep stoppers slightly loosened. Air from with in the chamber of the sterlise should be ejected allowing steam pressure to rise.Preparation of Plating Medium and Pouring2.3.3. Prepare growth medium in accordance with the composition indicated in 2.2.2.3.4. Melt the required amount of medium in boiling water or by exposure to following steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough medium which will be used with in 3h.Re-sterlisation of the medium may cause partial precipitation of ingredients.2.3.5. When holding time is les than 30 min, promptly cool the melted medium to about 45°C, and store until used, in a water bath or incubator at 43 to 45°C. introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the petridish at 42 to 44°C into each plate. Gently lift the cover of the dish just enough to pour in the medium, Sterlise the lips of the medium containers by exposure to flame.a. immediately before pouring.b. periodically during pouring, andc. when pouring

is complete for each batch of plates, if portions of melted medium remain in containers and are to be used without subsequent and are to be used without subsequent sterilization for pouring additional plates. As each plate is poured thoroughly mix the medium with test portions in the Petri dish.2.3.6. By rotating and tilting the dish and without splashing the medium over edge, spread the medium evenly over the bottom of the plate. Provided conditions so that the medium solidifies with reasonable promptness (5-10 min) before removing the plates from level surface.

3. Preparation of Serial Dilutions for Plate Counts. - 3.1. Dispense 30 g of Inoculant to 270 ml of sterile distilled or demineralized water and shake for 10 min on a reciprocal shaker or homogeniser. Make serial dilutions up to 10 (10) Take 0.2 ml or suitable aliquots of 10(6) to 10 (9) dilutions using sterile pipette and deliver to Petri dishes containing set medium as given in 2.1 and spread it uniformly. Invert the plates and promptly place them in the incubator.

4. Incubation of Plates. - Label the plates and incubate at 28 +/-2°C for 3 to 5 days for fast growing Rhizobia and 5 to 10 days slow growing ones

4.2Colony Counting aids.Count the colonies with the aid of magnifying lens under uniform and properly controlled artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally. Avoid mistaking particles of un-dissolved medium or precipitated matter in plates for pin-point colonies. The distinguish colonies form dirt, specks and other foreign matter, examine doubtful objects carefully.4.3Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies which absorb Congo red and stand out as reddish colonies. Rhizobium stands out as white, translucent, glistening and elevated colonies. Count such colony numbers and calculate figures in terms of per liter, of carrier. Also check for freedom from contamination at 10 (8) dilution.

1. B. Method of Analysis of Azotobactor Bio-Fertiliser

1. Apparatus - same as 1 of 1A of part B of schedule-II.

2. Reagents. - 2.1. Medium

Use a plating medium of the following composition

Agar	20 g
Sucrose (C12 H2 Ol 1)	20.0 g
Ferric sulphate (Fe2 (S04)2)	0.1 g
Dibasic potassium phosphate (K2HP04)	1.0 g

Magnesium sulphate (MgS04, 7H20)	0.5 g
Sodium Chloride (NaCl)	0.5 g
Calcium carbonate (CaC02)	2.0 g
Sodium Molybdate (Na2 MO 04)	0.005 gm
Distilled water 1000 ml	pH 6.8 to 7.2

2.2Sterlising & preparation procedure for plates:Same as 2.3.1 and 2.3.2 of 1 A of Part B of schedule-IIPreparation of Plating Medium and PouringSame as 2.3.3, 2.3.4, 2.3.5 & 2.3.6 of 1 A of part B schedule-II.

3. Preparation of Serial Dilutions for Plate Counts. - Dispense 30 g of Inoculant to 270 ml of sterile distilled water and shake for 10 min on a reciprocal shaker. Make serials dilutions up to 1010Take 0.2 ml or suitable aliquots of 106 to 109 dilutions using sterile pipettes and deliver to Petri dishes containing set medium as given in 2.1 and spread it uniformly. Invert the plates and promptly place them in the incubator.

4. Incubation of Plates. - 4.1. Label the plates and incubate at 28+/ - 3°C for 4 to 6 days.

4.2Colony counting aidsSame as 4.2 of 1 A of Part B of Schedule-IIAzotobactor chrococcum colonies are gummy raised with or without striations, viscous and often sticky. The pigmentation varies from very light brown to black. Count the colony number and observe the cyst formation as given below and calculate number per grant of the carrier material.Grow the vegetative cells at 30 C on Burks agar medium comprising sucrose 20 g, dipotassium hydrogen phosphate 0.64 g, dihydrogen potassium phosphate 0.20 g; sodium chloride 0.20 g; calcium sulphate 0.05 g, sodium molybdate 0.001 g; ferric sulphate 0.003 g. agar 20 g and distilled water 1.000 ml. Look for vegetative cells after 18 to 24 h either by simple staining method or through a phase contrast microscope.Grow the cyst cells on Burks agar medium as given above with 0.3 percent n-butanol in place of the carbon source. Look for cyst formation after 4 to 5 days incubation.

1.

C. Method of Analysis of Azospirillum Bio-Fertiliser

1. Apparatus. - same as 1 A of Part B of schedule II

2. Reagents. - 2.1. Medium

Use a plating medium of the following composition

Malic acid	5.0 g

Potassium hydroxide	4.0 g
Di-potassium hydrogen phosphate	0.5 g
Ferrous sulphate	0.05 g
Manganese sulphate	0.01 g
Magnesium sulphate	0.1 g
Sodium chloride	0.2 g
Calcium chloride	0.1 g
Sodium Molybdate	0.002 g
Distilled water	1000ml
Boromothymol blue (0.5% alcoholic solution)	2.0 m.
Agar	1.7 g
PH adjusted to	6.5 - 7.0

2.2Sterlising & preparation procedure for plates. - Same as 2.3.1 and 2.3.2 of 1 A of Part B of schedule - IIPreparation of Plating Medium and Pouring. - Same as 2.3.3, 2.3.4, 2.3.5 & 2.3.6 of 1 A of part B of schedule - II.

3. Preparation Of Serial Dilutions for Plate Counts. - Same as IB of part B of schedule-II

4. Incubation of Plates. - 4.1. Label the plates and incubate at 28+/ - 3°C for 4 to 6 days.

4.2Colony counting aids: Same as 4.2 of 1 A of part B of schedule-IICountingCounting the tubes or plates which have turned blue in colour after inoculation and ascertain the presence of pellicles in undisturbed medium. To determine usual contamination on the same examine doubtful objects carefully.Count all plates/ tubes which have turned blue and consider them for the purpose of calculation. Count such type of tubes/ plates and tally this court with MPN table Annexure-E to get the number of cells per gram of the carrier.

Azospirillum Count/g of carrier =| MPN table value x Dilution levelDry mass of product

1. Apparatus. - same as 1 A of Part B of schedule-I

2. Reagents. - 2.1. Medium

Use a plating medium of the following composition:

Glucose	10.0 g
Tri-calcium phosphate	5.0 g
Ammonium sulphate	0.5 g
Magnesium sulphate	0.1 g

Sodium Chloride	0.2 g
Yeast extract	0.5 g
Manganese sulphate	Trace
Ferrous sulphate	Trace
Distilled water	1000 ml
Agar	15.0 g

PH adjusted to 7 +/- 0.2

2.2Sterlising & preparation procedure for plates. - Same as 2.3.1 and 2.3.2 of 1 A of Part B of schedule-IIPreparation of Plating Medium and Pouring. - Same as 2.3.3, 2.3.4, 2.3.5 & 2.3.6 of 1 A of part B of schedule-II.

3. Preparation of Serial Dilution for Plate Counts. - Same as 1 B of part B of schedule-II

4. Incubation of Plates. - 4.1. Label the plates and incubate at 28+/ - 3°C for 4 to 6 days.

4.2Colony counting aids: Same as 4.2 of 1 A of part B of schedule - IICountingCount the total number of colonies on the plates including colonies with solubilisation zone with the help of a colony counter.Methods for counting solubilisation zones. - (a) Take 10 g of PSBI (BF) in 90 ml in water(b)Make a ten fold dilution series up to 107(c)Take 0.2 ml aliquote of 105 to 107 dilution using sterile pipettes and delivered to Petri dishes containing pikowskeyi media.(d)Spread it uniformly, Invert the plates and incubate them up to 2 weeks at 28+/- 2°C.(e)Count the colonies showing hallow cones and measure their diameter. Minimum acceptable zone is 10 mm in diameter.Guidelines of Maintenance and Preparation of Culture and Quality Control at Broth Stage.

1. Rhizobium:

1. Maintenance of pure cultures. - 1.1. Maintain pure cultures of rhizobia on yeast extract mannitol agar (YEMA) slants of the following composition.

Mannitol	10.0 g
Potassium hydrogen phosphate (k2HP04)	0.5 g
Magnesium sulphate (MgS04 7H20)	0.2 g
Sodium chloride (NaCl)	0.1 g
Calcium Carbonate (Ca C03)	1.0 g
Yeast extract	1.0 g
Agar	18.0 g
Distilled water	1 liter
pH	6-8-7.0

1.2Transfer a loopful of the pure culture to each of the slants aseptically in an inoculation room and incubate at 28+/- 2°C for 3 to 10 days depending upon the species of Rhizobium. Always keep pure cultures at 4°C.

2. Preparation of Inoculum Cultures

2.1Prepare yeast monnitol broth of the composition as given in 1.1. minus the agar.2.2Transfer a loopfull of the culture into a 100/250 ml conical flasks containing the broth. Incumbate the flasks at 28+- 2°C on the rotary shaker for 2 to 6 days.

4. Quality Control Tests Recommended at Broth Stage. - 4.1. Qualitative Tests

4.1.1. Check for freedom from visible contaminants4.1.2. The pH of the bacterial broth shall normally be between 6.5 and 7.54.1.3. Smear and Gram stain4.1.3.1. Reagentsa. Ammonium oxalate crystal violet stain weigh 0.2 g of crystal violet and dissolve in 20 ml of 95 percent ethyl alcohol. Dissolve separately 0.8 g of ammonium oxalate in 80 ml of distilled water. Mix the two solutions and fitter through a filter paper.b. Iodine solution

Iodine	1.00 g
Potassium Iodide	2.00 g
Distilled water	300 ml

Weigh the ingredients and dissolve in water. Filter through a filter paper.c. Erythrosine

Erythrosine	1.00 g
Phenol	5.00 g
Distilled water	100 ml

Weight the ingredients, dissolve in distilled water and filter through a filter paper.4.1.3.2. ProcedurePrepare a smear on a claim microscope slide, fix over a flame by gentle and intermittent heating, air cool and flood with ammonium oxalate crystal violet stain for 1 min. After removing the excess of ammonium oxalate crystal violet, wash the slide under a gentle stream of running tap water. Flood the slide with iodine solution for half of minute remove excess stain wash with 95 percent ethyl alcohol and finally wash under a gentle stream of running tap water. Flood the slide with erythrosine stain for about 3 min, wash under a gentle stream of running tap water and dry between the folds of a filter paper. Examine the slide under a compound microscope using an oil immersion objective.Note. - A smear prepared from undiluted broth should be free from Gram positive cells. The presence of a few gram positive cells in occasional fields which may be due to dead cells in the medium maybe disregarded.4.1.4. Absence of Growth on Glucose -Peptone AgarThe composition of the glucose - peptone agar is as follows:

Glucose	10.0 g
Peptone	20.0 g
Sodium chloride (NaCI)	5.0
Agar (IS 6850}	15.0

Distilled water	1000 ml
Bromocresol purple	10 ml of
ethyl alcohol solution	1.6 persons
pH	7.2

Note. - When a loopful of the broth is streaked into this medium and incubated at 28+/- 2°C for 24 h, the purple violet colour of the medium (due to the indicator bromocresol purple) shall not change. If the colour changes to yellow (acidic reaction) or blue (alkaline reaction) the broth is grossly contaminated Hence, the broth should be rejected.4.1.5. Streak on yeast Extract monnitol Agar with Congo RedWhen a loopful of broth culture is streaked to plate of this medium and incubated at 28 +/- 2°C for 3 to 10 days, it shall show colonies of bacteria with growth characteristics same as that of the pure culture use in the preparation of the broth, Other wise, the broth should be rejected.4.2Quantitative Test4.2.1. Viable or Plate CountsSerially dilute one milliliter of the broth to obtain dilutions of the order of 106 to 109. Plate 0.2 ml aliquots of the dilutions on YEMA plates and incubate at 28 +/- 2°C for 2 to 6 days, depending on the species of Rhizobium. The counts of viable Rhizobium in the final broth from shake culture or fermentors shall be not less than 108 to 109 cells/ml. Other wise, the broth should be rejected.

2. Azotobactor

1. Maintenance of pure cultures.

1.1Maintain pure cultures of Azotobactor on slants of the following composition

Agar	20 gm
Sucrose	20 gm
Ferric Sulphate	0.1
Dibasic Potassium Phosphate	1.0 gm
Magnesium Sulphate	0.5 gm
Calcium carbonate	2.0 gm
Sodium Molybdate	0.005 gm
Distilliled water	1000 ml
PH	6.8 to 7.2

1.2Transfer a loopful the pure culture to each of agar slants aseptically in an inoculation room and incubate at 28 +/- 2°C for 3 to 10 days depending upon the species of Azotobactor. Always keep culture pure cultures at 5°C.

2. Preparation of inoculum culture. - 2.1 Prepare Jonsons media broth of the composition as given in 1.1. minus the agar

2.2Transfer a loop full of the culture into 100ml/250 ml, conical flask containing the broth. Incubate the flasks at 28 +/- 2°C on a rotary shaker for 2 to 6 days.

3. Quality control Tests recommended at broth stage.

3.1Qualitative test. - 3.1.1 Check for free from contaminants by preparing slide and observing under microscope.3.1.2The pH by bacterial broth shall normally be between 6.5 to 7.03.1.3Gram staining test shall be carried out as described in 4.1.3, 4.1.3.1 and 4.1.3.2 of Rhizobium of this standard.3.2Quantitative test3.2.1. Viable cell count: same as 4.2. of IB of part B of Schedule II

3. Azospirillum

1. Maintenance of pure cultures. - 1.1. Maintenance of pure cultures of Azospirillum on nitrogen free bromothymol blue medium and maintain as semi solid medium as described in 2.1 of this standard 1C of part B of Schedule II

1.2Transfer a loopful of pure culture to each of the agar culture tube aseptically in an inoculation room and incubate 28 +/- 2°C for three days and keep in undisturbed.Always keep pure culture below 5°C.

2. Preparation of Inoculum culture. - Inoculum culture shall be prepared as described in 2.1, 2.2 of Rhizobium of this standard. ,,

3. Quality Control Test recommended at Broth Stage

3.1Qualitative Test3.1.1. Check for free from contaminants by preparing slide and observing under microscope.3.1.2. The pH of bacterial broth shall normally be between 6.5 to 7.0.3.1.3. Gram staining test shall be carried out as described in 4.1. 3., and 4.1.3.1 & 4.1.3.2. of Rhizobium of this standard.3.1.4. See the colour change in the media after 24 hours from inoculation. The colour will change from green to blue.3.1.5. Watch the pellicle just below the surface of the media. It checked on the third day after keeping inoculated broth undisturbed.3.2Quantitative Test3.2.1. Most Probable Number (MPN) as given in Annexure-E. The counts of Azosprillium in the final broth from shake culture or fermentors shall be not less than 108 to 109 cells / ml. Other wise the broth should be rejected.

4. Phosphate Solubilizing Bacterial Inoculant (PSBI)

1. Maintenance of pure cultures. - 1.1. Maintain pure culture of PSBI on the medium as described in 2.1 of 1 D of part B of schedule- II in the form of slants

1.2Transfer a loopful of pure culture to each of the agar slants aseptically in an inoculation room and incubate at 28 + /- 2°C for three days. Always keep pure culture below 5°C.

2. Preparation of Inoculum culture. - Inoculum culture shall be prepared as described in 2.1, 2.2. of Rhizobium changing the media composition as mentioned in 2.1 of this standard.

3. Quality control test reo amended at Broth Stage. - 3.1. Qualitative Test

3.1.1. Check for free from visible contaminants by microscope and observing solubilisation zones.3.1.2. The pH of bacterial broth shall normally be between 6.5 to 7.0.3.1.3. Gram staining test shall be carried out as described in 4.1.3,4.1.3.1, and 4.1.3.2 of Rhizobium.3.2Quantitative Test3.2.1Viable cell count serially dilute one milli liter of broth plate 0.2. ml aliquots of the dilutions on pikowkyasi media (as given in 2.1 of 1 D of Part B of Schedule II) plates and incubate at 28 +/- 2°C for 2 to 6 days. The counts of PSBI in the final broth from shake culture or fermentors shall be not less than 108 to 109 cells/ml. Otherwise, the broth should be rejectedAnnexure-ADetermination of PH

1. Make suspension of 20 g of the ASI into 50 ml of distilled water and shake on a rotary shaker for 2 hours. Filter this suspension and determine the pH of the filterate with the held of pH meter.

Annexure-BDetermination of Moisture of Bio-Fertiliser Packets (Method)2.1Heat 10 g of sample for 12-16 hours in an air over at 100-105°C, in desiccator and weigh. The loss in weight represents the moisture. Calculate the moisture percentage on air dry weight basis, by multiplying the loss in weight by ten.Annexure-CDetermination of Soluble Phosphorus Using Ascorbic Acid.Principle. - Soluble phosphorus from hetropoly molybdophosphate complex with molybdate ions which on reduction produces blue colour measured at 840 to 880 nm. Considering the higher stability of the ascorbic acid, easiness to handle, higher tolerance to the concentration of interfering ions, possibilities to use it with all type of acids and higher stability of the developed colour (10 to 60 min), ascorbic acid instead of stannous chloride is now - a-days used as the reducing agent for the hetropoly molybdophosphate complex formed by the soluble phosphate ions on addition of ammonium molybdate solution.apparatus. - Spectrophotometer capable of transmission measurements at 840 to 880 nm.Reagents. - Ammonium Molybdate(NH4)6 M07024 4H20)

1. - Ascorbic acid

P - Nitrophenol

4NH.

2S.

04.

Preparation of Reagents. - Sulphomolybdic acid.Take 20 g of Ammonium molybdate and dissolve in 300 ml of distilled waterAdd slowly 450 ml of 10 N2 S04.Cool the above mixture and add 100 ml of 0.5 percent solution of Antimonypotassium tartrate.Cool and make the volume to one litre. Store in glass bottle away from direct sunlight.Preparation of mixed Reagent. - Add 1.5 gm of L - ascorbic acid in 100 ml of the above stock solution and mix. Add 5 ml of this solution to develop colour. Mixed regent is to be prepared fresh as it does not keep for more than 24 h.Procedure. - Weigh the required material in a 100 ml conical flask.Add 50 ml of extractant and shake it for 30 min on rotary shaker.Filter the suspension through whatman filter paper No. 40. If the filterate is coloured than add a tea spoon of Darco-60 (activated phosphorus free carbon) reshake and filter.Take a known aliquot (5 to 25 ml) of the extract in a 50 ml volumetric flask.Add 5 drops of P-nitrophenol indicator (1.5 percent solution in Water) and adjust the pH of the extract between 2 and 3 with the help of NH2S04. The yellow colour will disappear when the pH of the solution becomes 3. Swirl gently to avoid loss of the solution along with the evolution of C04.When the C02 evolution has subsided. Wash down the neck of the flask and dilute the solution to about 40 ml.Add 5 ml of the sulphomolybdic acid mixed reagent containing ascorbic acid Swril the content and make up the volume.Measure the transmission after 30 min. at 880 nm using red filter. The blue colour developed remains stable up to 60 minutes.Record the concentration of Phosphorus (P) in the extract from the standard curve and calculate the concentration of soluble phosphorus as follows.Calculation. - (a) Weight of the substance taken = xg(b)Volume of the extractant added=50 ml(c)Volume of the extract taken for p=y ml determination.(d)Volume made after colour developed = 50 ml(e)Reading from the standard curve = z ppm against percent transmission recorded.(f)Soluble phosphorus.

Percent p =| ZX50X10 -6X5O X 100Y,X

Preparation of standard curve. - Prepared standard curve using 0.1 to 0.6 ppm P in 50 ml volumetric flask plot the standard curve by taking concentration of soluble P on x-axis and percent T on Y-axis using a semi-log graph paper. It is a straight line relationship between the soluble P and percent T when plotted on semi-log graph paper.Annexure-DDirection for Use of Bio FertilisersRhizobium

1. The contents of the packet are sufficient for seeds to be sown in the area indicated on the package.

2. Use only for the leguminous crops mentioned, before the expiry date and do not expose to direct sun light or heat.

3. Mix the inoculants with the seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seeds under shade over clean paper or gunny bag and sow immediately.

This is not a chemical fertiliser and hence do not mix inoculated seeds or RI with chemical

fertilisers.Azotobactor

1. Use only for the non-leguminous crops and before the expiry date.

2. The contents of the packet are sufficient for seeds to be sown in 0.4 hectare.

3. This is not a chemical fertilizer, hence do not mix it or the inoculated planting material with chemical fertilizers or pesticides.

4. Use for the crops specified on the packet.

5. Do not expose to direct sunlight or heat.

Azospirillum

1. The contents of the packet are sufficient enough to the given area to be broadcasted or given seedlings for root dipping depending on the specified crops as denoted in the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seed under shade over clean surface gunny bag and sow them immediately.

2. Use only for the crops mentioned paddy, Azospirillum brasillense for other crops like millets, etc use crop specific strain. Use before the expiry date and do expose to direct sun light or heat.

3. ASI is not a chemical fertilizer hence do not mix inoculated seeds or ASI with agrochemicals.

Phosphate Solubilising Bacteria.

1. The contents of the packet are sufficient enough to the given area to be broadcast or given seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed cost. Dry the inoculated seed under shade over clean dry paper organic bag and sow them immediately.

2. In order to solubilise fixed soil phosphate use PSBI for all type of crops. Use before the expiry date and do not expose to direct sunlight or heat.

3. PSBI is not chemical fertiliser hence do not mix inoculated seeds or PSBI with agrochemicals.