# A N2Africa universal logo representing inoculant quality assurance

Milestone 3.3.3

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## N2Africa

**Putting nitrogen fixation to work for smallholder farmers in Africa**

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# 1 Summary

This report specifically addresses ***Milestone 3.3.3.*** *Universal logo representing quality assurance standards adopted among cooperating laboratories (month 6, year 3).* Laboratory procedures backstopping this report were covered under Milestone 3.3.1 (N2Africa Report 019, Bala 2011). A common logo is proposed that bears three different grades of inoculant that reflect various competencies in manufacture (Figure 1). These grades span the standards of several countries and permit a stepwise approach that permits inoculant manufacturers to meet a minimal standard upon market entry and then recognize improvement in their product with time.


**Figure 1.1: Proposed QA label**

# 2 Background

This Milestone is part of the larger Activity 3.3 intended to formulate improved inoculant products and develop cost-effective production methods, including quality assurance procedures. It is built upon earlier tasks that 1) develop quality assurance (QA) protocols based on African capacities and international existing standards (MS 3.3.1, month 6 of year 1) and 2) develop cost effective inoculant production methods including fermentation technologies, carrier selection, inoculant formulation, and enhanced shelf life (MS 3.3.2, month 12 of year 2). In so doing, the cost of inoculant manufacture would be lowered and the efficacy of inoculation products improved, resulting in more affordable and higher quality inoculants that are distributed through better targeted production and marketing strategies. The universal logo stands as a quality assurance mechanism that these earlier milestones were satisfied and informing stockists and farmers that a given inoculant contains sufficient live rhizobia to result in an inoculation response. The N2Africa universal logo is intended to be voluntary, and to reflect different grades of inoculant based upon different manufacturing technologies and prudent processing.

# 3 Global industry standards

Standards and regulatory compliance mechanisms vary between countries, with generally higher standards in more developed settings (Table 3.1) This is due in part because inoculant standards must correspond to what is achievable by competent manufacturers. Across countries, industry compliance may be tightly regulated (e.g. Argentina, Canada and France), voluntarily monitored (Australia, Brazil and Thailand) or non-existent through reliance upon competitive market forces (USA). Examples from several countries follow.

**Table 3.1: Inoculant quality thresholds and regulation in selected countries**

| Country | Quality threshold (rhizobia per g) | Comment | Citation |
|---|---|---|---|
| Argentina | $> 1 \times 10^8$ | Regulated by law, closely monitored | Benintende 2010 |
| Australia | $> 1 \times 10^9$ | 10-fold reduction allowed during sale | Herridge et al. 2002 |
| Brazil | $> 1 \times 10^9$ | Recently increased from $10^8$ per g or ml | Hungaria et al. 2006 |
| Canada | $\approx 1 \times 10^9$ | Regulated by law, closely monitored | Rennie 1991 |
| France | $\approx 1 \times 10^8$ | Regulated by law, closely monitored | Wadoux 1991 |
| India | $> 1\text{-}5 \times 10^8$ | 10-fold reduction allowed during sale | Thompson 1984 |
| Kenya | $> 1 \times 10^9$ | Pending approval of draft Biofertizer Act | Wafullah 2013 |
| New Zealand | $> 1\text{-}5 \times 10^8$ | Higher standards by some producers | Callaghan (no date) |
| Thailand | $\approx 1 \times 10^8$ | Monitoring based upon seed counts | Boonkerd 1991 |
| USA | not in place | Quality maintained by strong competition | Herridge et al. 2002 |

### Argentina

This country has a comprehensive regulatory structure that proscribes inoculant label information, the number of viable rhizobia ($>10^8$ rhizobia per g or ml) and presence of contaminants. Nonetheless, 20% of the labels were defective, mostly related to expiry dates being removed or altered. Product quality is fair with 76% of samples reportedly exceeding the $10^8$ threshold (Benintende 2010).

### Australia

At the earliest stages of Australian regulation of non-sterile inoculants, $10^7$-$10^8$ rhizobia per g was considered adequate and afforded two months' expiry. Inoculants with $>10^8$ /g were allowed six months to expiry (Thompson 1984). South Africa set similar standards (Van Rensburg and Strijdom, 1974). The advent of sterilized, peat-based inoculants led to increase to a threshold $10^9$ per g and requirement that all inoculants must be free of contamination at $10^6$. Inoculants in Australia are allowed a 10-fold drop before expiry so inoculants passing $10^9$ per g in the factory may be sold by retailers until falling below $10^8$ per gram. Later, independent regulatory monitoring by the Australian Legume Inoculant Research Unit was extended to testing of initial broth, freshly manufactured inoculant, and the product on sale. While participation in monitoring is voluntary, producers are required to use specific strains for different legumes (Herridge et al. 2002).

### Brazil

Industrial scale production of legume inoculants in Brazil started in the 1960s but was not regulated by the Federal Government until 1975. The initial standard was $10^7$ cells per g, a concentration that would supply only 7,000 cells per soyabean seed. In 1982, this threshold was raised to $10^8$ viable cells per g in the factory and $10^7$ at the retail shelf with penalties imposed for carrying substandard product. Standards were raised again in 2004 to a minimum concentration of $10^9$ viable cells per g or ml, providing 1.2 million rhizobia per seed, with six months expiry. Inoculants must not contain greater than $10^5$ contaminants. (Hungria et al., 2006) In this way, Brazil represents a stepwise guide to how legume inoculants may be effectively regulated.

### Canada

Inoculants are regulated through the country's Fertilizer Act and products must be approved through Agriculture Canada. Standards are based upon seed counts and vary with seed size (Rennie 1991). Agriculture Canada tests a large number of product samples per year and reported 87% compliance but incidence of excess contaminants, including human pathogens, were also noted (Olsen et al. 1995; Herridge et al. 2002).

**France**

France established legislation regulating inoculants based upon proof of efficacy, use of sterile carriers and delivery of at least $1 \times 10^6$ rhizobia/soyabean seed, about $5 \times 10^8$ per g (Wadoux 1991). All inoculants offered for sale must be registered, tested by INRA in Dijon and contain strains aligned to particular legume species.

**India**

The Indian Standards Institution has somewhat lower thresholds of $10^8$ rhizobia per g at manufacture and $10^7$ at expiry (Thompson 1984). Even at these lower thresholds, compliance with these standards is irregular.

**Kenya**

There is only one inoculant producer in Kenya and it has set its quality standards of $10^9$ viable rhizobia per gram. BIOFIX is prepared from sugarcane filter mud carrier that harbours contaminants even after autoclaving, so populations of fungi remain high in the final product. Legume inoculants are covered in the pending Biofertilizer Act and should contain $>10^9$ rhizobia and $<10^6$ contaminants per g.

**New Zealand**

Peat cultures have shelf lives of six months and must not fall below $10^8$ viable rhizobia per gram. They are tested under the Plant Diseases Certification scheme. Many years ago, some producers maintained higher standards, such as Biological Laboratories Ltd. (Auckland) that assured greater than $5 \times 10^8$ rhizobia per g and maintained their own rigorous laboratory, greenhouse and field testing (Callaghan 1958).

**Thailand**

Thailand established robust national standards and independent testing by the Thai Department of Agriculture (Boonkerd 1991), again with standards based upon delivery of $10^5$–$10^6$ rhizobia per soyabean seed.

**USA**

Regulatory compliance of inoculants is not considered necessary in the USA due to competitive marketing by a large number of producers and the sophistication of customer farmers. Basically, inferior products are quickly forced from the market. Larger producers maintain rigorous product testing and labelling, and receive few or no customer complaints (Stew Smith, personal communication) but others have occasionally reported inferior products (Herridge et al. 2002; Olsen et al. 1995).

Effective quality control measures are prerequisite to maintaining high quality legume inoculants, and experience suggests that many producers are unwilling to conduct rigorous internal quality control programs. This is due in part because farmers cannot judge product quality when purchased, but once inferior products are obtained farmers lose confidence in the technology. For this reason, legume inoculant quality must be protected through some independent regulatory or industry association mechanism that once implemented benefits both farmers and competent manufacturers (Olsen et al. 1995). Clearly, many models are available for the establishment and enforcement of legume inoculant industry standards, and the challenge to Africa rests in identifying a system the assures farmers' purchases are not wasted but that standards are not set too high that manufacturers are discouraged or forced out of the market.

# 4 The universal logo

Three grades of the N2Africa universal logo of legume inoculant quality are proposed (Figure 2). These inoculants are solid, prepared from an organic carrier and may either be finely powdered to < 300 mesh for application to seed or pelleted for application to soil. Differences in grade are largely the result of contamination within the carrier prior to mixing with broth, the broth delivery method and competency in manufacture. Lower grades have shorter shelf lives,

**Grade B.** This grade contains greater than 100 million viable rhizobia and less than 10 million contaminants per gram of inoculant recovered from the factory. It generally results from bulk mixing of heat treated carrier and rhizobial broth, and curing on large trays prior to bagging and must be prepared and marketed immediately before the growing season. Its grade is similar to the minimum standards of Argentina, India, South Africa and Thailand (Table 3.1). This grade is seen as a starting point to higher quality production and is applicable to the pilot manufacture of inoculants by N2Africa cooperators in DR Congo, Malawi and Rwanda.

**Grade A.** This grade contains greater than 1 billion viable rhizobia and less than 1 million contaminants per gram of inoculant recovered from the factory. It results from incomplete sterilization of carrier with either bulk mixing or bag injection delivery systems. The number of viable rhizobia is allowed to drop 10-fold (to $10^8$ rhizobia/g) during product distribution and marketing, but is only good for one season. Its grade is similar to the minimum standards of Australia, and Brazil, and those proposed in the Biofertilizer Act of Kenya (Table 3.1).

**Grade AA.** This grade contains greater than 1 billion viable rhizobia with no detectable contaminants at $10^{-5}$ dilution. This grade changes little in quality after one year and is allowed to be carried over by distributors between two seasons (but is best sold the season it is produced). It results from complete sterilization of carrier by gamma or e-beam irradiation with a bag injection delivery system. Its grade is similar to the minimum standards of Canada and France and careful monitoring is required to assure compliance with its microbial population thresholds (Table 3.1).

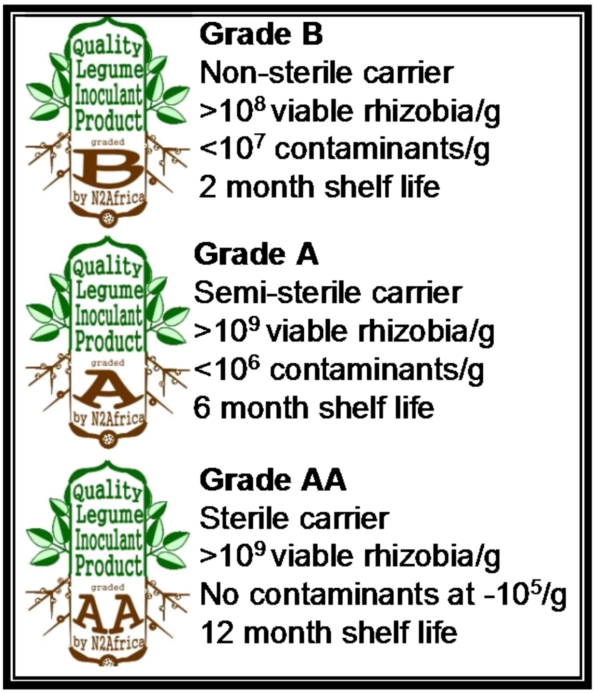


**Figure 4.1: The proposed grades and quality standards of legume inoculants under the N2Africa universal logo**

**Inoculant product labelling**

The information required on the legume inoculant package should include:

1. Name of the crops for which the inoculant is intended
2. Guarantees of number of live rhizobia and contaminants per gram
3. Expiration date beyond which the product cannot be used
4. Lot number for quality control feedback
5. Instructions for use (translated into local languages)
6. Net weight of inoculant
7. Trade name, manufacturer and address
8. Necessary storage conditions

# 5 Implications for N2Africa program activities and planning

A variety of important, complementary roles exist for the public sector, the private sector and international agency in the development, promotion and delivery of legume inoculants (**Table 5.1**). The public sector must assume the lead in providing technical assistance in areas of rhizobiology, extension and quality control in a manner that both stimulates private investment and safeguards inoculant customers (Singleton et al. 1997). Of importance to the N2Africa Program is the role of international agency, particularly in developing capacities and methods in rhizobiology, coordinating the activities of national programs and helping to bridge the gaps between public sector services and private sector needs. While admittedly international agency cannot operate in all places, it can help set precedents for collaboratin between the public and private sectors and establish incentive for improved performance by the inoculant sector. The universal logo of inoculant quality, and this report, play a small role in the scope of these services, but its fuller context is developed under Milestone 3.4.5 that formalizes strategic alliances between research centres and the private sector for inoculant production and use (month 12 year 4).

Thompson (1984) and others maintain that in the presence of contaminating organisms, plate counts alone are inadequate and estimates by MPN must also be conducted. On the other hand, it is more difficult to conduct plant dilution counts in developing countries of the tropics owing to a lack of suitable facilities (e.g. plant growth rooms) because this procedure is less reliably performed in the greenhouse. Indeed, just one "skip" at a low dilution superficially yields a much lower population estimate (Woomer et al. 1988). What is most important is that farmers do not receive a substandard product and that quality assessors are not expected to adhere to out-of-reach procedures. The N2Africa quality endorsement is somewhat limited in another way because it does not consider the

**Table 5.1: Relationships among the public sector, private business and international agents in the production, quality assurance and improvement of legume inoculants**

| Function | Public Sector | Private Sector | International agency |
|---|---|---|---|
| *Rhizobium* | | | |
| collection & curation | Maintain national culture collections. | Access industry standards, maintain mother cultures. | Bio-prospect, initiate & coordinate national culture collections. |
| strain evaluation | Conduct routine strain evaluation, identify candidate elite strains. | Compare new elite strains to industry standards. | Identify, characterize and exchange elite strains. |
| *Inoculant* | | | |
| formulation | Support pilot facilities & access to product components. | Develop new formulations and products. | Assist in streamlining production costs and methods. |
| manufacture | Compile and release commodity statistics. | Produce inoculants in profitable, cost effective manner. | Evaluate and exchange different production approaches. |
| standards | Establish standards for labelling and contents. | Develop processes and competencies to comply with standards. | Compare standards & provide guidelines for compliance. |
| use | Conduct extension campaigns on inoculation. | Establish branded demonstrations & participate in shows. | Develop and translate extension materials on inoculant use. |
| regulation | Monitor product quality and report compliance. | Label product properly & conduct quality assurance. | Design protocols for product testing. |
| trade | Reduce obstacles to cross-border trade. | Develop distribution networks and product advertising. | Provide market information and policy support. |

presence of specific strains for given legumes or problems associated with genetic instability affecting infectiveness and effectiveness, or even colony dimorphism (Herridge et al. 2002).

Some of the rhizobiology laboratories working with N2Africa in countries that lack commercial inoculant production have initiated pilot production, including those in DR Congo, Malawi and Rwanda. These laboratories must strive to produce inoculants that meet at least Grade B in quality so that their goals of establishing the technical feasibility, demonstrating inoculation to farmers and raising awareness of benefits from inoculation to policymakers may be achieved. Herridge et al. (2002) caution that few pilot facilities have launched larger private sector operations because of the research, rather than commercial, focus of those activities. It is crucial that public sector pilot factories transition from test manufacturing inoculant to providing a fuller range of technical support services to private sector investors entering production (Singleton et al. 1997).

Lastly, one may challenge the relevance of this report given that N2Africa has yet to assist commercial inoculant producers in Africa to enter input supply markets. The Program intended to spawn three such enterprises, one in each regional hub (Milestone 3.4.3), but failed to do so because these funds were redirected to upgrading national rhizobiology laboratories. This change was deemed necessary because public sector capacities required to backstop commercial activities were not in place, and in part because N2Africa scientists themselves were not particularly skilled in industrial inoculant production, and the learning curve is steep. One exception was the collaboration with MEA Ltd. in Kenya that launched its BIOFIX product just as the program started. Collaboration with MEA led to a substantially improved product that now ranks as Grade B, occasionally higher, in our universal logo based on routine independent testing by the Nairobi MIRCEN (Table 5.2). Note that the difficulty in controlling contaminants, mostly fungi, due to incomplete sterilization of the filter mud carrier prior to broth injection. Another entry point is the initiation of the NoduMax inoculant factory in the IITA Business Incubation Platform. The universal logo Grade A is included within the package design, with the possibility of achieving Grade AA depending to the cost effectiveness of more advanced production technologies (see MS 1.3.3 Report). During the proposed N2Africa Phase 2 it is hoped that the universal logo will find greater utility.

**Table 5.2: Results of quality control testing of BIOFIX inoculants during 2012 by the UoN MIRCEN (S. Kisamuli, personal communication)**

| Batch | Host | Rhizobia x $10^9$ | Contaminants x $10^6$ |
|---|---|---|---|
| 13031202G | Greengram | 4.17 | 0.75 |
| 13041202B | Bean | 12.33 | 4.33 |
| 170312P | Groundnut | 8.33 | 4.67 |
| 24031202S | Soyabean | 8.50 | 2.67 |
| 14041202S | Soyabean | 7.83 | 1.25 |
| 02031202S | Soyabean | 7.33 | 2.33 |
| 130812023S | Soyabean | 4.67 | 3.33 |
| 30812028B | Bean | 1.25 | 2.50 |
| Average | | 6.80 | 2.73 |
| CV (%) | | 49.5% | 50.0% |

# 6 Conclusions and recommendations

A candidate logo and accompanying grading system was designed and distributed to many N2Africa Program cooperators for discussion (Figure 1.1). A way forward was proposed that includes escalating grade criteria that permits entry into markets and stepwise improvement over time (e.g. Grade B = $>10^8$ rhizobia, contaminants $< 10^7$, Grade A = $>10^9$ rhizobia, contaminants $<10^6$, and Grade AA = $>10^9$ rhizobia, no contaminants, see Figure 2). Also considered was a "side grade" for inclusion of adhesive agent (e.g. +) or (in)sufficient or (in)correct information on packet, but this is not described in detail within this report. This standard requires approval from inoculant producers, importers and perhaps endorsement from the African Association for Biological Nitrogen Fixation at its next meeting (2014).

So how can the N2Africa universal logo be employed? In some ways it is a good idea that did not succeed because the inoculant production envisaged under Milestone 3.4.3 (450,000 packets produced over three years) did not occur. Without these packets there was no new products to test and nowhere to affix the logo. There are the pilot products under development in DRC, Malawi, Rwanda and next in Nigeria, and those engaged in these efforts should be offered the use of the inoculant logo. The proposed N2Africa Phase 2 intends to carry forward in this area by developing Standard Operating Procedures (SOP) for quality evaluation of inoculants, and this logo and its grades could be modified to reflect and formalize the routine results from this SOP (Activity 4.8. Develop standard operating procedures for the production, quality control and application of rhizobium inoculants). With these considerations the following recommendations are offered:

1. Test the quality thresholds of rhizobia and contaminants against additional inoculants, including those produced in the pilot factories and imported from elsewhere, and adjust these grades to competency of manufacture and effectiveness of product. To date these proposed standards are based primarily upon the scientific literature and only two products, BIOFIX (Kenya) which rates Grade B and LegumeFix that offers Grade AA.
2. Explore the inverse relationship between rhizobium and contaminants within inoculants, and develop procedures that better sterilize carriers and extend product shelf lives as a component of N2Africa Phase 2 Activity 4.8.
3. Offer the logos to the pilot inoculant producers in DRC, Malawi and Rwanda as a means to ensure that their new products at least meet Grade B.
4. Reevaluate the concept and need for the universal logo and its grade standards within the context of the policy briefs under development under Milestone 3.5.1 that is due at the end of the program (October 2013).

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# List of project reports

1. N2Africa Steering Committee Terms of Reference
2. Policy on advanced training grants
3. Rhizobia Strain Isolation and Characterisation Protocol
4. Detailed country-by-country access plan for P and other agro-minerals
5. Workshop Report: Training of Master Trainers on Legume and Inoculant Technologies (Kisumu Hotel, Kisumu, Kenya-24-28 May 2010)
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22. Production of seed for local distribution by farming communities engaged in the project
23. A report documenting the involvement of women in at least 50% of all farmer-related activities
24. Participatory development of indicators for monitoring and evaluating progress with project activities and their impact
25. Suitable multi-purpose forage and tree legumes for intensive smallholder meat and dairy industries in East and Central Africa N2Africa mandate areas
26. A revised manual for rhizobium methods and standard protocols available on the project website
27. Update on Inoculant production by cooperating laboratories
28. Legume Seed Acquired for Dissemination in the Project Impact Zones
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31. Existing rhizobiology laboratories upgraded
32. N2Africa Baseline report
33. N2Africa Annual country reports 2011

34. Facilitating large-scale dissemination of Biological Nitrogen Fixation
35. Dissemination tools produced
36. Linking legume farmers to markets
37. The role of AGRA and other partners in the project defined and co-funding/financing options for scale-up of inoculum (banks, AGRA, industry) identified
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39. Quantifying the impact of the N2Africa project on Biological Nitrogen Fixation
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# Partners involved in the N2Africa project

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