

Chapter 2

Global Standards in Seed Health Testing

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Abstract Routine seed health testing is carried out in most countries for seed certification and plant quarantine. However, the majority of seed health tests used throughout the world have never been subject to rigorous validation. A fully validated test provides for analytical sensitivity, reproducibility and repeatability. Discrepancies between testing methods can occur, leading to costly phytosanitary disputes or liability claims. These issues can be avoided by working toward a system of universally accepted, standardized testing methods on a global level. To ensure that seed health tests are standardized and give reliable and reproducible results in accordance with the given specifications of the test methods, methods should go through a peer review system and/or collaborative study among laboratories. Three primary organizations publish standardized seed health tests: the International Seed Testing Association (ISTA), the International Seed Health Initiative (ISHI), and the U.S. National Seed Health System (NSHS). In 1957, the ISTA Plant Disease Committee (PDC) established a comparative seed health testing program to standardize techniques for detection of seed-borne pathogens. In 1993, the Seed Health Committee (SHC, formerly the PDC) began development of published guidelines for comparative testing. All ISTA validated methods are published in the International Rules for Seed Testing. Additionally, some ISHI-Veg methods have been accepted as ISTA Rules and as Standards by the NSHS. The procedures followed by ISTA, ISHI and the NSHS to achieve global standards in seed health testing are discussed.

Keywords International Seed Health Initiative • ISHI • International Seed Testing Association • ISTA • National Seed Health System • NSHS • Seed-borne pathogens • Validation

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1 Historical Review

Hiltner discussed methods for determining seed health of seed in germination tests at the second International Seed Testing Congress in Münster and Wageningen in 1910 (Mathur and Jørgensen 2002). However, according to Agarwal and Sinclair (1987), the first seed health testing laboratory in the world was only established in 1918 at the Government Seed Testing Station, Wageningen, the Netherlands with Lucie Doyer being appointed as the first official seed pathologist in 1919. The International Seed Testing Association (ISTA) was formed in 1924 at the fourth International Seed Testing Congress in Cambridge and at the next congress in 1928, L. Doyer reported on the activities of the “Plant Disease Committee” (PDC) which had split from the “Committee on Determinations of Genuineness of Variety in Field” established in 1924 (Mathur and Jørgensen 2002). The first recorded seed health methodologies were published by Dorogin in 1923 as *Instructions for testing seeds to determine the presence of fungus diseases at seed control stations* (Agarwal and Sinclair 1987) and Doyer in 1938 as the *Manual for the determination of seed-borne diseases* (Doyer 1938). In 1954 the European Plant Protection Organisation (EPPO), a regional organization established in accordance with the International Plant Protection Convention (IPPC) of 1951, sponsored by FAO, published a report of the first working party on seed-borne diseases titled *Danger from seed-borne diseases. A practical approach to the problem of international safeguard* (EPPO 1954). According to Mathur and Jørgensen (2002), the dominating factor influencing the development of seed health testing was the increased awareness of plant quarantine problems and extra pressure was put on ISTA to develop standard methods for such testing. A.J. Skolko, chairman of the ISTA PDC, presented the first report on comparative seed health testing of oats, flax, cabbage, wheat, barley and beet in 1956 at the 11th ISTA congress and the decision was made that the PDC would continue with comparative testing. P. Paul F.M. deNeergaard (Fig. 2.1), chairman of the ISTA PDC from 1956 to 1974, established the first comparative seed health testing programme to standardise



Fig. 2.1 P. Paul F.M. deNeergaard (Anon 1985)

techniques for the detection of seed-borne pathogens in 1957 (Mathur and Jørgensen 2002). This involved providing a number of independent seed scientists with referee seed samples and comparing results at an annual workshop of which the first was held in Cambridge, United Kingdom, in 1958 (Neergaard 1970). Neergaard played a leading role in the organisation of international cooperative testing of laboratory procedures for detection of pathogens on and in seed aiming at attaining uniformity and international standardisation (Anon 1985). In the last of three joint EPPO/ISTA Working Parties in London in July 1965, health certification of seeds for export was discussed and specified seed health testing methods developed and standardized by ISTA were recommended for quarantine inspection of seed lots (Neergaard 1970). Although the first ISTA *International Rules for Seed Testing* were published in 1931 (Steiner et al. 2008), the first specific seed health methods in the ISTA rules were only introduced in 1966 (Mathur and Jørgensen 2002; Muschick 2010). In 1998, Sheppard and Wesseling published a guide for comparative testing of methods for the detection of seed-borne pathogens. This was the basis for the *ISTA handbook of method validation for the detection of seed-borne pathogens* published by Sheppard and Cockerell (Fig. 2.2) in 2000. At that time Sheppard was the chairman of the then called Seed Health Committee (SHC). Based on this publication the Method Validation Working group was established in 2002 to develop the ISTA Method Validation Programme which came into force in 2007 and now applies to all seed quality testing (Hampton 2005, 2007). The term “method validation” is new in seed testing. According to Steiner et al. (2008) it was previously called “method standardization” or “method elaboration”.

The International Seed Health Initiative (ISHI) also develops, evaluates and disseminates information on seed health test protocols. The International Seed Health Initiative for vegetables (ISHI-Veg) was established in 1994, herbage (ISHI-H) in 1997 and field crops (ISHI-F) in 1998, however, the latter two have not been active with only one method approved by ISHI-F (ISF 2013). ISHI-Veg started as an initiative of the vegetable seed industry when Dutch and French seed companies started a project on monitoring seed health in 1993. The USA, Japan and Israel joined the initiative which then represented more than 75 % of the world's



Fig. 2.2 Jim W. Sheppard and Valerie Cockerell

vegetable seed supply and in 2000 the International Seed Federation (ISF) took over the secretariat and financial administration (ISF 2013).

The Association of Official Seed Analysts (AOSA) formed in 1908, is an organization of member laboratories across the United States and Canada that establishes and publishes seed testing rules in the *AOSA Rules for Testing Seeds* (AOSA 2013). Although AOSA does have a seed pathology research sub-committee, it is the National Seed Health System (NSHS) that is involved in the evaluation of seed health methods. The NSHS, a program authorized by the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS), was established in 1999 and published in the Federal Register on 18 July 2001 to come into effect on 17 August 2001 (Federal Register 2001). The NSHS is administered by the Iowa State University Seed Science Centre (NSHS 2013).

2 Seed Health Test Method Evaluation and Validation

2.1 ISTA

ISTA method validation critically examines a seed quality test to ensure that the description of the method is clear and complete and that the procedure gives accurate, reproducible and repeatable results (Hampton 2007). The operation of the formal ISTA Method Validation process relies on co-operation between the Technical Committee Coordinator (TCord) and the Technical Committee (TCOM) (in the case of seed health testing, the SHC). The TCord and the ISTA Secretariat support the TCOM by keeping records of the process and providing the TCOM chair with information they require to monitor the process. The validation process is described and published on the ISTA website as the *ISTA Method Validation for Seed Testing* (ISTA 2007) and the *Standard Operating Procedure for Method Validation Process Administration* (Hampton 2009; ISTA 2013) and is summarized below. ISTA also provides guidelines for ISTA rules proposals and instructions for authors and reviewers of draft test plans and validation reports on their website (ISTA 2013). Details and progress of method validation projects, which are the responsibility of the TCOM, are presented in an Excel workbook on the ISTA website.

ISTA seed health validated methods have been through multi-laboratory collaborative studies however, in some situations (for example: the addition of a new species to an existing method) ISTA has introduced its Peer-Validation Programme for the validation of methods by laboratories working with only one or two others (ISTA 2007). The validation process proceeds as follows (Fig. 2.3): If the SHC decides to proceed with a submitted test plan for a seed health test, two technical reviewers are appointed and the Statistics Committee (STA) appoints a statistical reviewer to review the test plan. The reviewed test plan is returned to the TCord.

The TCord forwards the reviews to the SHC who either rejects the test plan or approves it with or without minor revisions. The applicant of the approved test plan acts as or appoints a test organiser who drives the validation study, analyses of results and produces a validation report which is first sent to the validation study participants before being sent to the TCord. The TCord forwards the validation report to the SHC and STA who appoint two technical reviewers and one statistical reviewer, respectively. The reviewers return their reviews and the validation report to the TCord who forwards them to the SHC. The SHC either rejects the final validation report (an ISTA Method Validation Report) which includes all results, statistical data and a working method based on the reviews or approves them with or without minor revisions. The SHC prepares the method for the rules proposal on the basis of the validated report and the method becomes an ISTA Rules proposal (ISTA 2013). The method is published on the ISTA website to coincide with announcements of Rules proposals to be voted on at the next ISTA Ordinary Meeting providing the ISTA voting delegate with all the documentation to make an informed decision. The method requires the acceptance by the ISTA membership by vote at an Ordinary Meeting before publication of the validated method in the ISTA Rules. There are currently more than 29 validated ISTA seed health methods (some having been validated for the same pathogen but on different substrates) and are published as 7-001a to 7-029 on the ISTA website and in Chap. 7 of the ISTA rules (ISTA 2013).

2.2 *ISHI-Veg*

The method validation procedure followed by ISHI-Veg (ISF 2013) is summarized below and in Fig. 2.4. The International Technical Group (ITG) is responsible for developing a seed health test plan. There are four ITGs within the Technical Co-ordination Groups (TCG) namely, roots, bulbs and leafy vegetables; bean, pea and brassica; cucurbits; and tomato and pepper, that meet every 9–11 months. A nominated representative represents each country in the ITG but can consist of more than one representative per country depending on the crop/pathogen combination. Each TCG chairperson co-ordinates and maintains the ISHI-Veg comparative test plans developed by the ITGs. The TCG chairperson also represents the TCG in the ISF Phytosanitary Committee and the Policy Co-ordination Group (PCG), which consists of one member each from the participating countries. The PCG determines the annual budget of ISHI-Veg, develops policy guidelines to assist the TCG, decides which of the reference test methods developed by the TCG appear on the ISF website in the ISHI-Veg Manual and determines the recommended sample size for each test method. The PCG chair reports to both the ISF Vegetable Crop Section and the Phytosanitary Committee at the annual ISF Congress. Through the presentation of a draft motion and its subsequent adoption by the Vegetable Section a position taken by ISHI-Veg can become the position of ISF (Fig. 2.4). The seed health testing methods were the basis for the position

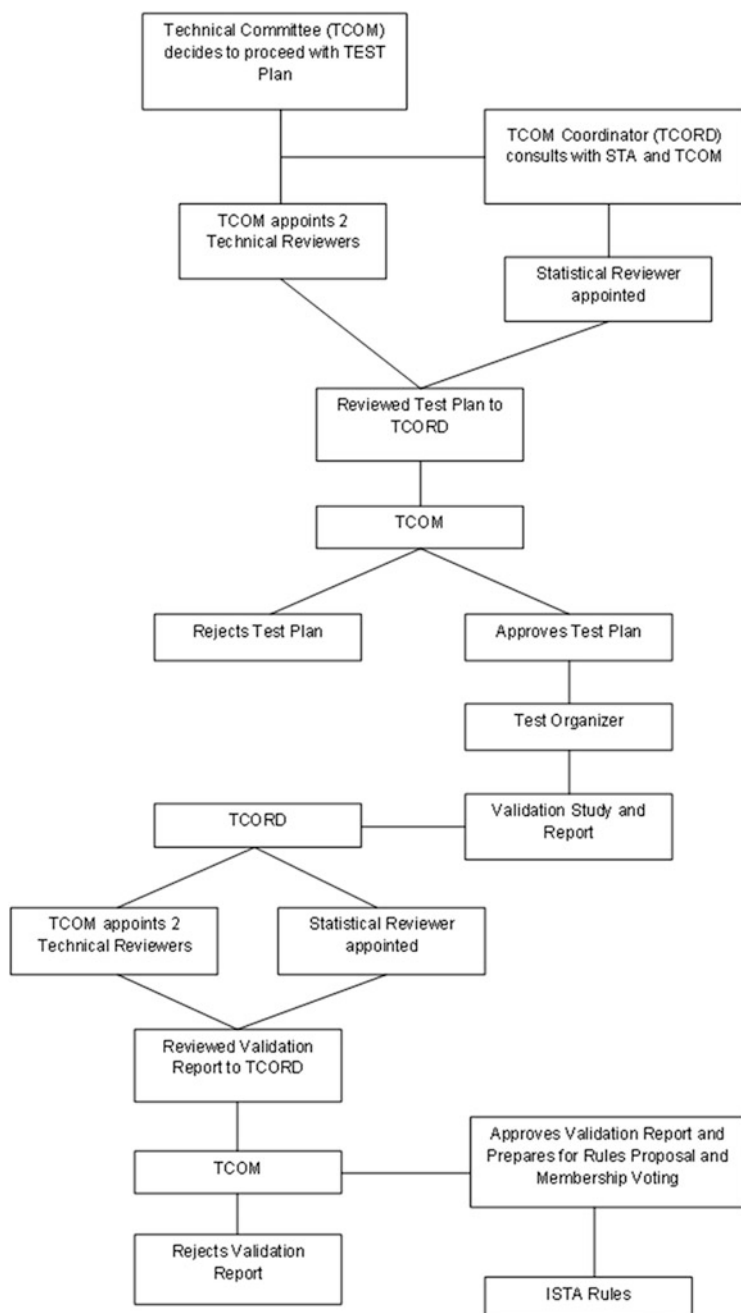


Fig. 2.3 The process followed by the Seed Health Committee of the International Seed Testing Association (ISTA) to validate a seed health test method

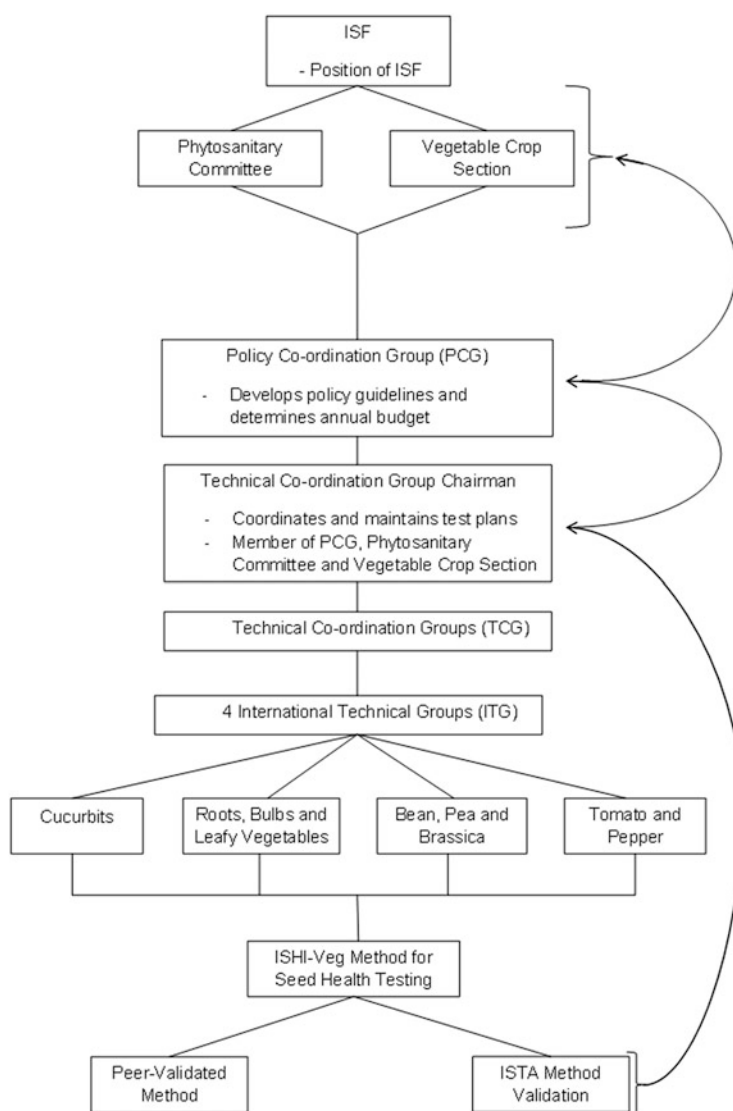


Fig. 2.4 The process followed by the International Seed Health Initiative for Vegetables (ISHI-Veg) of the International Seed Federation (ISF) to validate a seed health test method

adopted by the vegetable seed sector in May 2006 titled *Guidelines for the use of seed health methods by the vegetable seed industry*, which was revised in 2010.

The TCGs are composed of seed health scientists from public and private sectors and are responsible for the establishment of reliable test methods that (1) are clear and reproducible; (2) are practical and feasible for routine testing by technical staff; (3) give results that are indisputable; (4) function as generally accepted reference

methods; (5) serve as legal references in court cases; (6) support the international seed industry in improving product quality and; (7) serve as documentation for phytosanitary certification according to IPPC guidelines (ISF 2013). For a method to be accepted by ISHI it must be described and available for public use or published in a peer-reviewed journal and must be approved by the TCG of the particular vegetable crop. The method then enters the validation process according to ISTA's Method Validation for Seed Testing (ISTA 2007) which includes a multi-laboratory comparative test of 6–8 company and public laboratories. The number of laboratories and samples are determined in the design of the test plan and if less than the six laboratories required by ISTA are used, the method may be accepted as a "Peer-Validated Method" and endorsed as an ISHI reference method. Data related to the validation of components of the method and the peer review are stored in the ISHI database (ISF 2013). Currently ISHI-Veg has 21 methods in their *Manual of Seed Health Testing Methods* of which 11 have been adopted as ISTA rules and 11 as NSHS standards (Munkvold 2009; ISF 2013).

2.3 NSHS

Reference Manual A. The reference manual for administration, procedures, and policies of the NSHS, published by the NSHS in 2000, describes the structure, administration, procedures, policies and working practices of the NSHS and also contains relevant documentation, forms and references for the NSHS (Federal Register 2001; NSHS 2013). The NSHS evaluation procedure of a new seed health testing method (NSHS 2013) is described below and in Fig. 2.5. The NSHS Administration Unit develops a technical package of scientific information on each host/pathogen combination. This includes published seed health tests, unpublished seed health tests in use, proprietary methodologies in use, data on test standardization by ISHI, ISTA or other organizations and relevant data on seed transmission or detection of the pathogen. This technical package is forwarded to a Technical Panel chosen from a number of seed health experts both nationally and internationally and from private and public sectors based on their area of expertise. Members of the Technical Panel volunteer to review existing and proposed new seed health testing methods and are approved by the Seed Technical Working Group (STWG). The Technical Panels use the following criteria to evaluate seed health test methods: (1) Empirical test data that determines the sensitivity, specificity, repeatability and reliability of the assay; (2) Comparative test data with already established methods; (3) Historical data of methods used in industry or academia and the record of number of uses or complaints and; (4) Other criteria which may have an impact on the recommendation for use of a test. Each Technical Panel reviewer prepares an individual report rating each of the methods evaluated by these criteria which is then submitted to the Technical Panel chair who prepares a summary technical report submitted to the STWG. The technical panel members use the following guidelines for rating the methodology(s):

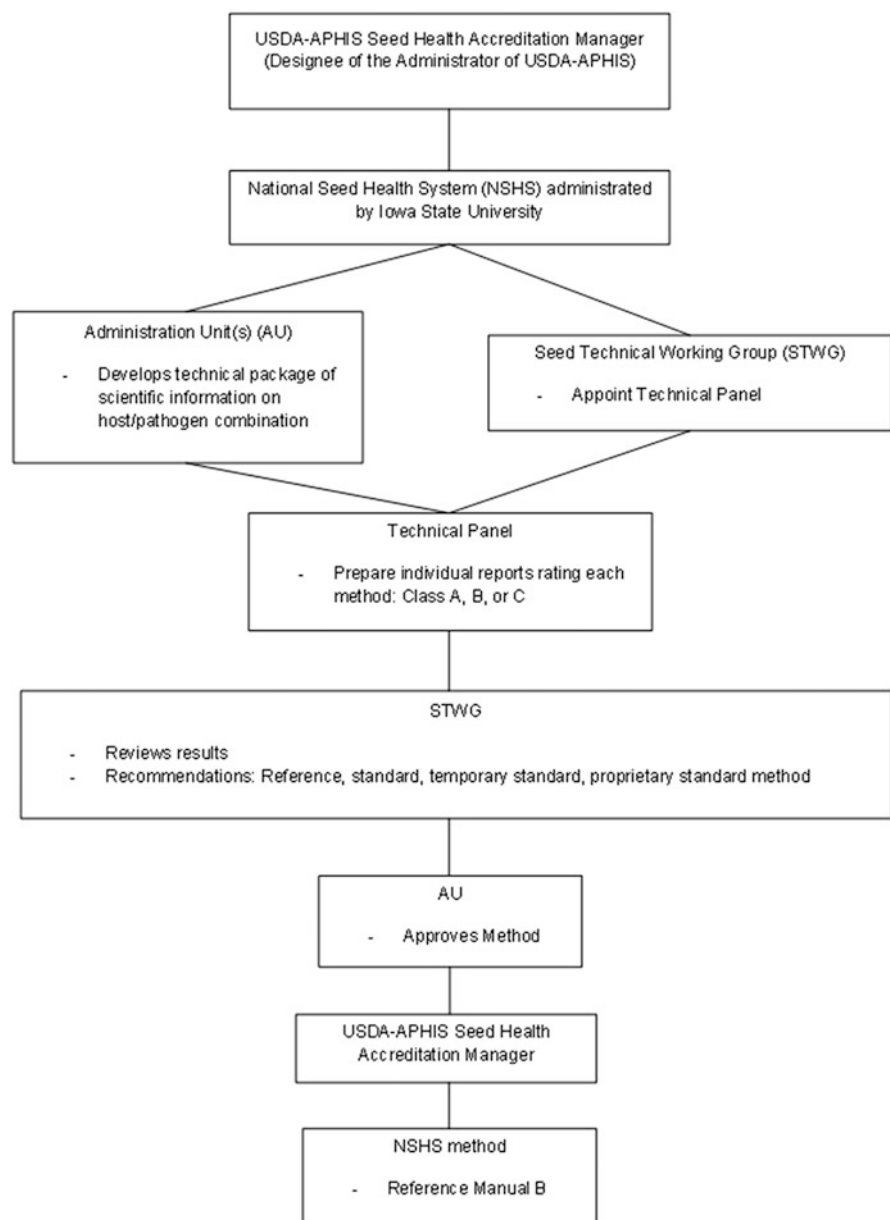


Fig. 2.5 The process followed by the United States of America National Seed Health System (NSHS) to evaluate a seed health test method

- Class A – The test or method acceptable as a standard test
- Class B – The test or method needs further research before acceptance as a standard test. This could be for improvement to the method itself or a recommendation for a comparative test with a known method
- Class C – The test should not be accepted as a standard test.

The STWG is responsible for reviewing the results and recommendations of the Technical Panel before forwarding them to the Administrator Unit for approval. There are four categories that the methods can be divided into:

1. Reference method – Recommended Class A and NSHS approved method accessible to all users
2. Standard method – Recommended Class A and NSHS approved method but method owner may recover royalties from use of the method
3. Temporary standard method – Recommended Class B and STWG approved for a defined period
4. Proprietary standard method – Comparable to an NSHS reference method and approved by the STWG.

All these methods may then be included in Reference Manual B for official use in phytosanitary certification following APHIS approval with exception of the proprietary standard method (NSHS 2013). *Reference Manual B. The reference manual for seed health testing and phytosanitary field inspection methods* published by the NSHS in 2001 contains the detailed seed health testing, seed sampling and inspection methods and procedures for the NSHS and is constantly updated (Federal Register 2001; NSHS 2013).

Conclusion

The procedures followed by the major role players in achieving global standards in seed health testing, namely ISTA, ISHI and the NSHS have been summarized. The procedures followed by the Seed Health Committee of ISTA and ISHI-Veg are very similar and are based on the validation procedure manual published by Sheppard and Cockerell (2000). ISTA and ISHI-Veg make use of collaborative multi-laboratory tests and elaborate statistical procedures and analyses to validate a new seed health test method. These procedures differ greatly from those followed by the NSHS. The NSHS appoints a panel that reviews scientific literature of existing seed health test methods to compare them with a new proposed method. There are no collaborative laboratory tests involved and hence, new methods are adopted and published by the NSHS in Reference Manual B far quicker than the adoption and publication of ISHI-Veg and ISTA methods. Methods adopted by ISHI-Veg and ISTA can be approved as NSHS methods but the reverse is not true unless collaborative multi-laboratory tests are conducted using the NSHS method. ISHI-Veg seed health methods often become ISTA methods.

(continued)

NSHS seed health methods are approved by the USDA-APHIS. In ISHI-Veg a new method is adopted by ISF, by both the ISF Vegetable Crop Section and the Phytosanitary Committee, after presentation at the annual ISF Congress. The new seed health method adoption process by ISTA involves several stages starting with the publication of all validation reports on the ISTA website and in *Seed Testing International* (ISTA 2013). The new method is sent out to the entire ISTA membership for perusal as part of the new methods to be adopted at the next ISTA ordinary meeting. At this meeting the new method is presented to the ISTA membership and can only be adopted as a new seed health method after a majority vote by each country's designated voting member. Furthermore, the seed health methods are reviewed every 5 years by the SHC and laboratories using the methods.

Although the global seed industry requires that the number of standardized seed health tests be exponentially increased, organizations involved with the standardization of new methods have to insure that the methods are validated and thus reproducible and repeatable by laboratories from all over the world. This is essential for the overall harmonization of phytosanitary regulations involving the movement of seed in international seed trade (Munkvold 2009).

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