



GMO Task Force Proficiency Test Working Group:

2009 Survey on proposed use of devitalized seed in ISTA's GMO Proficiency Test program

Background: ISTA's GMO Proficiency Testing Program is growing as more labs participate each year. The amount of seed material required for each round is increasing and material used must be of highest purity for preparation of samples distributed to participating labs. The material must be procured by the ISTA Secretariat and tested to verify purity and identity prior to preparing the PT samples. With each round, obtaining GM and non-GM seed of sufficient amount and quality required for each PT is becoming more and more difficult. Alternatives to the use of samples prepared from whole, viable seed have been proposed, but, it is not known what impact this would have on labs that participate in the ISTA program - including those currently ISTA Accredited for GMO testing and those considering seeking accreditation. The purpose of this survey was to determine what impact, if any, use of material other than whole, viable seed would have on this program. The results of the survey are below.

These results will be considered in future planning for ISTA's GMO TF PT Program. On behalf of the GMO TF and GMO PT Working group, I would like to extend sincere thanks to laboratories who took the time to participate and complete our survey.

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Survey Questions and Responses:

1. What technologies are currently used in your lab use for GMO testing? (38 respondents)

7/38 Lateral Flow Strip	(18.4%)
5/38 ELISA	(13.2%)
29/38 PCR (Gel based)	(76.3%)
31/38 Real Time PCR	(81.6%)
2/38 Herbicide Bioassay	(5.3%)
4/38 Microarray	(7.9%)
5/38 Other: see below:	(10.5%)

- hybridity system, oil composition in OSR
- competitor's trait and other in-house traits
- plants (canola, sugar beet potato etc.) from deliberate releases in GMO seed analysis
- PCR fluorescence based

Observations:

37/38 responding labs are currently testing for GM Traits (97.4%)



28/38 responding labs are using more than one technology (2-6 different technologies) (76.3%)

Most used technologies are PCR based (76.3% of responding labs use Gel-based PCR and 81.4% use Real Time PCR)

2/38 responding labs use Herbicide bioassay – both also use other technologies – for 1, these are protein based, for the other protein and DNA based.

2. What traits are currently tested for in your lab? (Please indicate using a tick if you are testing for low level presence (e.g. AP) or Trait Purity)

Insect resistance:	34/38 (89.5%) Low level presence	14/38 (36.8%) trait purity
Herbicide resistance:	33/38 (86.9%) Low level presence	15/38 (39.5%) trait purity
Other: See below:	13/38 (34.2%) Low level presence	5/38 (13.2%) trait purity

- End-point Taqman PCR
- PCR-based technologies
- Different combination of traits, stacked or not.
- 1% AP screens of herb & insect trait testing
- Event specific Soy Mon 40-3-2
- CaMV 35S promoter & Agrobacterium tumefaciens NOS terminator
- Promoter and terminator regions
- few construct specific modifications
- antibiotic resistance genes, promotor, terminators
- antibiotic resistance, fatty acid composition
- starch potato (EH92-527-1)
- antibiotic resistance

Observations:

LLP testing is being done by 86.9% (HR) to 89.5% (HR) of responding labs

Trait purity testing is being done by 36.8% (IR) to 39.5% (HR) of responding labs

GM testing using methods other than trait detection for LLP and Purity is being done by 34.2%(IR) to 13.2% (HR) of responding labs

3. Do the method(s) used by your lab require use of whole, viable seed? (Check any that apply)

-> % based on # of respondents using a given technology

7/38 using Lateral Flow Know	2/7 (28.6%)Yes	4/7 (57.1%) No	1/7(14.3%)	Don't
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5/38 using ELISA Know	1/5 (20%) Yes	4/5 (80.0%) No	0/5 (0%)	Don't
29/38 using PCR (Gel based) Know	5/29(17.2%)Yes	21/29 (72.4%) No	3/29 (10.3%)	Don't
31/38 using RT-PCR Know	3/31 (9.7%) Yes	26/31 (83.9%) No	2/38 (6.5%)	Don't
2/38 using Herbicide Bioassay Know	2/2 (100%) Yes	0/2 (0%) No	0/2 (0%)	Don't
3/38 using Microarray (1 response) Know	0/3 (0%) Yes	1/3 (33.3%) No	0/3 (0%)	Don't
4/38 using Other (3 responses) Know	0/4 (0%) Yes	3/4 (75.0%) No	0/4 (0%)	Don't

- End-point Taqman PCR
- PCR fluorescence based
- the use of whole seeds is required regarding the limit of detection of the method that may be different for each lab (for qualitative methods). And also in my case **as I perform sub-sampling strategy**. Regarding viable seeds, that depends on the method used I guess. If is heat, may be a problem for protein based methods. May be you can prepare whole seeds samples for labs like our (that perform sub-sampling strategies), and send grinded samples to labs that perform RT PCR. Sending grinded material is commonly done by other organisations that provide interlaboratory tests, and is well accepted by other accreditation bodies
- Cry 3A 90 **seed trait purity requires seedlings**
- The DNA extraction is performed on seed flour. This means that the seeds to be tested are destroyed
- ** we know our methods will work on ground material – flour, cracked seed, meals, as well as on some foods, but we do not know about other types of devitalized seeds – such as high heat/pressure treatments, chemical or UV treatments, etc.
- * PCR methods can be used even with non-viable seeds. The presence of broken seeds may represent a challenge (use **of sub-sampling strategy**, preparation of working samples of a given size, e.g. 3.000 seeds)
- There are no legal constraints with specific instructions regarding the use of whole seeds for GMO testing.

Observations:

The need for whole viable seed to perform GMO testing is related to the testing approach and technology employed by individual labs

Responses were mixed with regard to need for whole viable seed for all technologies except those using Herbicide Bioassay.

For labs performing herbicide bioassay (2/38 respondents) – only viable, whole seed can be used.



Overall:

16.7% of respondents stated their methods required whole, viable seed

75.6% of respondents stated their methods did not require whole, viable seed

7.7% of respondents did not know if their method required whole, viable seed

**4. Will the method(s) used in your lab for GMO testing work when applied to devitalized seed?
 (Tick ✓ all that apply)**

-> % based on # of respondents using a given technology

7/38 use Lateral Flow (6 responses)	4/7 (57.1%)	Yes	1/7 (14.3%)	No	1/7 (14.3%)	Don't Know
5/38 use ELISA	5/5 (100%)	Yes	0/5 (0%)	No	0/5 (0%)	Don't Know
29/38 use PCR (Gel based)	18/29 (62.1%)	Yes	2/29 (6.9%)	No	9/29 (32.3%)	Don't Know
31/38 use RT-PCR	19/38 (50.0%)	Yes	2/38 (5.3%)	No	10/38 (26.3%)	Don't Know
2/38 use Herbicide Bioassay	0/2 (0%)	Yes	2/2 (100%)	No	0/2 (0%)	Don't Know
3/38 use Microarray (1 response)	1/3 (33.3%)	Yes	0/3 (0%)	No	0/3 (0%)	Don't Know
4/38 use Other (3 responses)	3/4 (75.0%)	Yes	0/4 (0%)	No	0/4 (0%)	Don't Know

Observations:

The success of methods applied to devitalized seed is related to the testing approach and technology employed by individual labs, but could vary depending on the method of devitalization:

Responses were mixed with regard to whether the method used will work with devitalized seed for all technologies except those using Herbicide Bioassay.

For labs performing herbicide bioassay (2/38 respondents) – 100% of respondents indicated their method would not work with devitalized seed

Unknown impacts were noted from 0-32.3% of respondents, mostly for those testing with PCR based methods (26.3 and 32.3% for RT-PCR and Gel based PCR respectively)

Overall:

64.9% of responses indicated their methods would work with devitalized seed

9.1% of responses indicated their methods would not work with devitalized seed

26% of responses indicated they did not know if their methods would work with devitalized seed

5. Please explain briefly any problems you may have testing devitalized seed: Comments:



- When testing for proteins (Lateral Flow Strip, ELISA) the target proteins need to be expressed. In devitalized seed there is no such expression therefore the detection is dependent on the longevity, or rate of decay, of the proteins synthesized prior to the devitalisation process. These variables are unknowns, and will most probably vary for different proteins; hence, I expect problems with protein testing methods of at the least some events.
- It will probably won't work when testing proteins. That also may depend on the treatment given to the seeds. If it is heat, it may not work. It is not in my experience, but I was told that heat would be no problem for testing DNA. I do not know if other methods are used
- DNA may be damaged by certain devitalization processes.
- Cry 3A 90 seed trait purity requires seedlings
- As long as that devitalized seeds have not an effect on the extracted DNA (quality, quantity) I think that we can use them.
- There will be no problem if the devitalized seeds allow normal DNA extraction in both amount and quality.
- If there is a lack of information about unknown material in devitalization process, there could be some unknown impact to PCR method.
- Note we use viable seedlings in the bioassay and then check any seedlings we are uncertain of using the strip tests or PCR, therefore for our system viable seed is essential.
- the method should not affect the DNA. Basis for our RT-PCR analysis. The treatment should not include possible compounds that may be inhibitory to RT-PCR technology and the traditional DNA extraction methods would not be able to eliminate.
- none
- the treatment of the seeds should not damage the DNA in the seeds
- not used before
- The DNA has to be in a condition that allows to amplify it.
- None foreseen – this lab uses DNA and protein based methods. Potentially, the methods we use could become less sensitive – for example if the devitalization method affected the DNA/proteins in such a way that they could not be detected, which could impact our ability to accurately quantitative content. We have validated our methods on devitalized seed (cracked seed/flours) but not on seed devitalized in any other manner. Thus, we cannot determine how they would be affected when applied to seed treated by another means. Devitalization by cracking or rough grind (i.e. not to fine flour) will be fine, while seeds treated by UV or other means could have degraded DNA/proteins. Determining the impact would have to be done through evaluation of the method on seeds that had been devitalized. Complete validation would be difficult, as there is more than one "method" for devitalization - each potentially impacting seed differently, thus representative devitalized seed from each method would have to be included to determine if there would be an impact or not. Obtaining reference material of this type of study is expected to be extremely difficult.
- No, because the method we use is PCR



- We don't have any experiences in testing devitalized seeds. May the devitalization (or some kind of) affect somehow the quantity of DNA in the devitalized seeds compared to the quantity of DNA of the viable seeds?
- If the GMO DNA is still intact then there should be no problems with our methods working. We have no problems with testing the use of de-vitalised seed.
- Check the effects on DNA before and after devitalisation process has taking place.
- Have no experience with devitalised seed

6. Is your lab currently ISTA Accredited for GMO Testing? 6/38 (15.8%) Yes **32 (84.2%) No**

7. Would the use of devitalized seed in ISTA's GMO PT program have an effect on your lab's position regarding ISTA Accreditation for GMO testing?

2/38 (5.3%) Yes **20/38 (52.6%) No** 13/38 (34.2%) Don't know

Respondents that indicated "yes":

- #7 – Accredited for GMO testing: no explanation given
- #15 – Not Accredited for GMO testing: We do not currently need to be accredited for GM testing but may want to be in the future and are proving competence by participating in the ISTA PT programme.

Other respondents:

- L Crop is now ISO/IEC 17025/2005 for trait purity and AP testing via bioassay and Immunoassay.
- It depends on the performance of the used methods on devitalized seeds
- We are currently accredited to a South African testing body and subscribe to a US-based proficiency testing programme and the use of devitalized seed has no effect on results obtained.
- At the moment in National Phytosanitary Laboratory are no further plans in near future for GMO accreditation (financial questions).
- MY LAB EXAMEN DISEASED AND VIRUSES INFECTED SEEDS FROM INSIDE OR OUTSIDE THE COUNTRY
- We suspect that our methods would still be able to detect and ID GM seeds. Quantification might be an issue if the devitalization process decreases the sensitivity of the test.
- If our current methods do not work with the de-vitalised material and if this was the preferred methods adopted by ISTA then we would need to re-consider our position. However, it is more than likely that our methods would be adapted to meet the ISTA requirements if there was a move to use de-vitalised seed.
- Have no experiences on devitalised seed.
- Have no experience with devitalised seed



8. If your lab is not currently ISTA Accredited for GMO testing, is this something that your lab is presently working towards, or would consider seeking in the future?

25/38 (65.8%) Yes 7/38 (18.4%) No

9. Would the use of devitalized seed for PT samples influence this decision?

4/38 (10.5%) Yes 27/38 (71.1%) No

Please explain briefly:

Respondents that indicated "yes":

- #14: Presently our LAB is collecting experience in PT, devitalized seed could help testing process make more easier (different permits and low).
- #15: no explanation given
- #16: no explanation given
- #18: no explanation given

Other respondents:

- As explained above, the results obtained are not affected by the fact that devitalized seed is used for a proficiency programme we already participate in. Not being accredited by ISTA is simply because we are already accredited by another body.
- we are DANAK accredited with ISO17025 and expect that to be the way we continue. We have too few seeds to ISTA accreditation, in addition to the DANAK
- The investment to become the ISTA accreditation is high compared to the number of seed samples we analyse. Moreover the procurement of the amount of seeds necessary to fulfill the PBA is difficult.
- We suspect that our methods would still be able to detect and ID GM seeds. Quantification might be an issue if the devitalization process decreases the sensitivity of the test.
- No, because the method we use is PCR

10. Please provide any additional comments or concerns regarding use of devitalized seed in the ISTA GMO PT, if any:

- Lab no.1: work with forest trees, mainly *Pinus sylvestris*, *P. contorta*, *Picea abies*. These are collected in known orchards or native stands with no GMO trees within. Don't think the GMO is of interest for the forestry yet or in the nearest future.
- If it has been demonstrated that the devitalization of seeds cannot affect the DNA extraction and RT-PCR, I consider that it is a good idea to have this type of material for proficiency test on gmo.



- I think that devitalisation methods that interfere with the germination process such as bleaching should be preferred over methods such as radiation that may have a more profound impact and affect the genome structure by inducing DNA breaks. The later may affect PCR results.
- It would be good to know how are the seed going to be devitalised.
- DNA damage leading to reduced LOD compared to viable cells
- once again the use of devitalized seeds depends on the influence that may be having on the quality and quantity of the extracted DNA.
- Existing information about devitalization of seed could be found and summarized in some official website (ISTA ?).
- The major focus of our GM work is trait purity not AP testing. In this approach bioassay is a useful/important/cost effective approach.
- for us it would be OK
- The DNA has to be in a condition that allows to amplify it.
- Obtaining material for validation of methods using devitalized seeds will be a challenge.
- It appears to be a good way to tackle the issues of Intellectual Property Rights and Product-stewardship and it may encourage Companies to release more reference materials in the form of seed. And if it works well it could encourage more participation in the Proficiency Tests.
- It is not necessary to germinate the seed samples for the methods currently used in our lab. So, for the time being, devitalization does not affect our methods. In addition, from the viewpoint of our domestic regulation applied to GMO, devitalized seeds would be preferable for us to import GM seeds if the traits have not been approved for the safety of biological diversity in our country.