Bhutan Agriculture and Food Regulatory Authority (BAFRA)

Ministry of Agriculture and Forests

First GMO Pilot Survey Report

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April –June 2017

ACKNOWLEDGEMENTS

We acknowledge the support from the European Union Global Climate Change Alliance - EU GCCA for providing Bhutan Agriculture and Food Regulatory Authority (BAFRA) with financial support of Nu. 0.3 Million for conducting the first nationwide pilot survey on GMOs/LMOs (Genetically Modified Organisms/Living Modified Organisms) following market based surveillance to study the presence of GMOs in food and feed, and field based appoach to survey whether GMOs are being cultivated in Bhutan.

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EXECUTIVE SUMMARY

Bhutan Agriculture and Food Regulatory Authority (BAFRA) under Ministry of Agriculture and Forests is the National Competent Authority to coordinate all biosecurity related activities in Bhutan. BAFRA is mandated to implement biosecurity regulatory measures (sanitary and phytosanitary measures) and food safety control system to protect the health and life of humans, plants and animals including the national biodiversity from risks of entry, establishment and spread of exotic pests and diseases, invasive alien species and genetically modified (GM) organisms.

The Biosafety Act of Bhutan 2015 requires the regulation and safety management of GM organisms and GM products in the Kingdom. The Biosafety Act prohibits the cultivation/environmental release of all GMOs. Also, the GMOs that are capable of reproducing are restricted for import, transit, intentional introduction, contained use, research and development. However, the Biosafety Act allows the import of GMOs and/or GM products for direct use as food, feed and processing (FFP) if its safety has been reviewed (By the technical Working Group on Biosafety) and is approved (by the National Biosafety Board of Bhutan). Any import of GM products for direct use as food, feed and processing (FFP) has to follow the prior approval system.

BAFRA has been designated as the National Competent Authority to regulate and coordinate all Biosafety related activities in the Kingdom. Therefore, BAFRA is required to conduct regular monitoring and surveillance to regulate the introduction of GMOs into Bhutan either in the form of grains or live plant materials of corn and soyabeans.

Accordingly, this cross-sectional pilot survey was conducted with the following objectives:

- *To study the presence of GM corn and soyabean in food and feed*
- *To survey whether GM corn and GM soyabean are being cultivated in Bhutan*
- *To draw up monitoring plans for field officials for surveillance of GMOs*
- *To create awareness for enforcing regulatory control of GMOs in line with the regulatory and safety management requirements as per the recently enacted Biosafety Act of Bhutan 2015.*

This cross-sectional pilot survey was conducted in 20 districts and 6 official entry points from May to June 2017. Grain samples were collected from retail and wholesale establishments including feed processing plants. The live plant samples (leaf) were collected from high-risk farms and nurseries. Simple or systematic random sampling techniques were used for the selection of establishments. A purposive risk-based sampling was used for selection of nurseries and fields.

Samples were tested using CP4 EPSPS (RR) LFS kits for Soyabean, Corn matrix, bulk soyabean grain, and for bulk corn grain. A total of 780 (82 corn leaves, 402 corn seed/grains, 19 soyabean leaves and 277 soyabean seed/grains) samples were tested. All the samples tested negative to the presence of GMOs. Therefore, this pilot study suggests that there is no presence of GMOs in these study areas. However, the BAFRA field offices will need to continue to conduct regular surveillance to detect presence of GMOs in the country.

BACKGROUND

Agricultural plants are often genetically modified by the insertion of foreign DNA material into their DNA sequence, resulting in expression of novel traits, typically herbicide tolerance or insect resistance. Current GMO production basically centers on four main crops: soyabeans, corn, cotton and mustard. Such genetic modification can be detected through the use of rapid test kits and advanced laboratory test using polymerase chain reaction (PCR).

The Biosafety Act of Bhutan 2015 requires the regulation and safety management of GM organisms and GM products in the Kingdom. Bhutan's agricultural policy is to promote organic and traditional agriculture production systems and to conserve native flora and fauna. Due to likely potential risks GMOs present to human and animal health, and to the environment, in the absence of a strong regulatory system, Bhutan's policies prohibit the cultivation or environmental release of all GMOs in the country.

Accordingly, the Biosafety Act prohibits the cultivation/environmental release of all GMOs. Furthermore, the GMOs that are capable of reproducing are restricted for import, transit, intentional introduction, contained use, research and development. The Biosafety Act allows the import of GMOs and/or GM products for direct use as food, feed and processing (FFP) if its safety has been reviewed (By the technical Working Group on Biosafety) and is approved (by the National Biosafety Board of Bhutan). Any import of GM products for direct use as food, feed and processing (FFP) has to follow the prior approval system.

Bhutan Agriculture and Food Regulatory Authority (BAFRA) under Ministry of Agriculture and Forests is the National Competent Authority to coordinate all biosecurity related activities in Bhutan. BAFRA is mandated to implement biosecurity regulatory measures (sanitary and phytosanitary measures) and food safety control system to protect the health and life of humans, plants and animals including the national biodiversity from risks of entry, establishment and spread of exotic pests and diseases, invasive alien species and genetically modified (GM) organisms.

BAFRA has been designated as the National Competent Authority to regulate and coordinate all Biosafety related activities in the Kingdom. Therefore, BAFRA is required to conduct regular monitoring and surveillance to regulate the introduction of GMOs into Bhutan either in the form of grains or live plant materials of corn and soyabeans. This is the first pilot survey to detect the presence of GMOs in the country. This study is conducted using the rapid test kits as a preliminary screening tool and were targeted to screen GM soyabean and GM corn, two most commonly used GMOs. Some of the samples were also randomly subjected to PCR test to confirm the results.

STUDY AIMS

The main aims and objectives of this cross-sectional pilot study are to:

- *study the presence of GM corn and soyabean in food and feed;*
- *survey whether GM corn and GM soyabean are being cultivated in Bhutan;*
- *draw up monitoring plans for field officials for surveillance of GMOs; and*
- *create awareness for enforcing regulatory control of GMOs in line with the regulatory and safety management requirements as per the recently enacted Biosafety Act of Bhutan 2015.*

MATERIALS AND METHODS

STUDY DESIGN

This cross-sectional pilot survey was conducted in 20 districts and 6 official entry points from May to June 2017.

SAMPLE SIZE AND SAMPLING METHOD

Since this is a pilot study, sample size was not estimated. In addition the number of samples tested was determined based on the number of test kits available. A summary of total Test Kits is as given below and the details of Test Kits distributed to the BAFRA offices are detailed in ANNEX I.

S.N	Supplier/ Manufacturer	Product Description	Pack Size	Price per unit	Quantity	Total
1	Amar Immunodiagnostics, India	AID 025-CP4 EPSPS (RR) LFS 680 kit for Soybean and Corn leaf/seed matrix	100 Strips/ Pack	Rs. 10000	20	200000
2	Amar Immunodiagnostics	AID 040- CP4EPSPS (RR) LFS 681 kit - Bulk soy bean grain	100strips/ Pack	Rs. 10000	5	50000
3	Amar Immunodiagnostics	AID 042- CP4EPSPS (RR) LFS 682 kit - Bulk corn grain	100strips/ Pack	Rs. 10000	5	50000

The Sampling Plan followe for the Pilot Survey using LF 680 (For corn/soyabean seed/leaf matrix), 681 (For bulk grain soybean) and LF 682 (bulk grain corn) is as presented in Sampling Plan A and B (attached as ANNEX II).

The Guide for assigning the Sample ID is attached as ANNEX III.

SAMPLE TESTING

Rapid test kits

To fully assess the characteristics of GMOs, the proteins expressed by those GMOs are quantified in a variety of matrices including, for example, plant tissues, diets, soil, water, and insects. When testing for GMOs, detection is achieved either through the inserted DNA or expressed protein translated from the novel DNA. The inserted DNA and its corresponding protein are found in all the cells of the plant. Thus, the seeds and/or plant tissue material (e.g. leaves) can be used for GMO testing using either rapid test kits and advance laboratory test such as PCR.

For this pilot survey, rapid test kits were used for screening. For the supply of the rapid tests kits, quotations were invited from 2 suppliers: Romer Labs, Singapore and Amar Immunodiagnostics, Hyderabad, India. As per the comparative analysis of Quotations from the two suppliers, it was recommended to consider procurement of CP4 EPSPS (*Agrobacterium tumefaciens strain CP4 and herbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase* enzyme) since it works on 2 plant matrices (soybean and corn) from Amar Immunodiagnostics, Hyderabad, India.

Amar Immunodiagnostics (http://www.amarimmunodiagnostics.com/#el2) is a leading provider of GMO testing kits in ELISA and lateral flow test format. These kits are used by leading seed companies in the world for testing genetically modified plants. These kits are also used by research institutes, university scientists and food processing industry to verify presence or absence of inserted trait in individual leaf/seed or bulk grain. The Company has been developing ELISA kits for European and American companies since last 8 years.

The testing for presence of GMO were conducted using following rapid test kits:

- AID 025: *CP4EPSPS (RR) Lateral Flow Strips (LFS 680) Test Kits are intended to be used for the qualitative detection of CP4EPSPS protein in individual Corn/Soybean leaf or seed samples.*
- AID 040: *CP4EPSPS (RR) Lateral Flow Strips (LFS 681) Test Kits for AP testing in bulk soyabean grains. The Kit detects one Roundup Ready soyabean in 1000 conventional corns (0.1%). The total incubation time of the assay is 10 minutes.*
- AID 042: *CP4EPSPS (RR) Lateral Flow Strips (LFS 682) Test Kits for AP testing in bulk corn grains. The Kit detects one Roundup Ready corn in 1000 conventional corns (0.1%). The total incubation time of the assay is 10 minutes.*

TEST PROTOCOL

Sample preparation, testing and interpretation of the results were done using protocol of the Test Kits. The protocols are attached as ANNEX IV.

Inspectors were trained for collection of samples and testing and handed over video test guide materials. Monitoring and guidance were provided by the focal officer from BAFRA Head Office.

The Test Reports were obtained from 20 BAFRA Dzongkhag offices with the following details:

1. Type of sample tested
2. Company/Nursery name
3. Location details
4. Sample details (Brand Name/Batch No/Manufacture Date)
5. Sample collection date
6. Name of sampler
7. Name of analyst
8. Sample testing date
9. Test kits used (LF 680/681/682)
10. Result
11. Remarks

RESULT AND DISCUSSION

A total of 780 samples were tested in 20 Dzongkhags in which 484 were corn samples and 296 were soyabean samples (Table 1). All sample tested negative in LF 680, LF 681 and LF 682 and also in few random samples tested using PCR.

The details of sample type tested are as below:

Corn Samples		**Soyabean Samples**	
Leaf	Seed/Grain	Leaf	Seed/Grain
82	402	19	277

Table 1. Test results of corn and soyabean samples tested for the presence of GMOs in 20 districts and 6 official entry points of Bhutan.

SUMMARY OF RESULTS									
S. N	Name of Dzongkhag	Corn Sample		Soyabean Sample		Total Samples (Corn + Soyabean Sample)	Test Kits Used		
		Positive	Negative	Positive	Negative		LF 680	LF 681	LF 682
1	*Tsirang*	*0*	*50*	*0*	*30*	*80*	√	-	-
2	*Samtse*	*0*	*36*	*0*	*0*	*36*	√	-	-
3	*Wangdue*	*0*	*12*	*0*	*15*	*27*	√	-	-
4	*Trongsa*	*0*	*10*	*0*	*6*	*16*	√	-	-
5	*Tashigang*	*0*	*4*	*0*	*6*	*10*	√	-	-
6	*Punakha*	*0*	*4*	*0*	*3*	*7*	√	-	-
7	*Paro*	*0*	*4*	*0*	*1*	*5*	√	-	-
8	*Lhuentse*	*0*	*2*	*0*	*2*	*4*	√	-	-
9	*Tashi Yangtse*	*0*	*2*	*0*	*0*	*2*	√	-	-
10	*Bumthang*	*0*	*2*	*0*	*0*	*2*	√	-	-
11	*Samdrup Jongkhar*	*0*	*150*	*0*	*70*	*220*	√	√	√
12	*Dagana*	*0*	*23*	*0*	*19*	*42*	√	-	-
13	*Phuentsholing*	*0*	*40*	*0*	*40*	*80*	√	√	√
14	*Gelephu*	*0*	*50*	*0*	*50*	*100*	√	-	√
15	*Zhemgang*	*0*	*2*	*0*	*2*	*4*	√	-	-
16	*Gasa*	*0*	*6*	*0*	*0*	*6*	√	-	-
17	*Haa*	*0*	*4*	*0*	*0*	*4*	√	-	-
18	*Thimphu*	*0*	*2*	*0*	*2*	*4*	√	√	√
19	*Mongar*	*0*	*50*	*0*	*5*	*55*	√	-	-
20	*Pema Gatshel*	*0*	*31*	*0*	*45*	*76*	√	-	-
TOTAL		***0***	***484***	***0***	***296***	***780***			

The result of this pilot survey suggests that there is no evidence of presence of GM corn or soyabean in these study areas. Although this pilot study was conducted in selected high-risk areas, it is not a representative study for the whole of country. In addition, the sample size is relatively less to have enough power to detect GMOs, if present in the country. A more comprehensive and larger representative study covering whole country needs to be conducted to confirm that the country is free of GMOs.

In the meanwhile, the BAFRA Dzongkhag and official entry-point offices need to continue to conduct regular surveillance using the rapid test kits in order to detect and prevent introduction of GMOs in the country.

CONCLUSION

In conclusion this pilot study suggested that there is no evidence of presence or introduction of GM corn or soyabeans either in the form of grains or live plant materials in these study areas. However, more comprehensive and respresentative study (either covering whole country and risk-based selection of areas) needs to be conducted to confirm free-status of the country for GM corn and soyabeans.

ANNEX I – DISTRIBUTION OF TEST KITS

S.N	BAFRA Offices	AID 025 LF 680	AID 040 LF 681	AID 042 LF 682	1.7ml micro--centrifuge vial
1	BAFRA, Trongsa	100 strips			100
2	BAFRA, Bumthang	100 strips			100
3	BAFRA, Mongar	100 strips			100
4	BAFRA, Lhuntse	100 strips			100
5	BAFRA, Tashigang	100 strips			100
6	BAFRA, Tashiyangtse	100 strips			100
7	BAFRA, Pemagatsel	100 strips			100
8	BAFRA, Samdrupjongkhar	100 strips			100
9	PAQS, Samdrupjongkhar		100 strips	100 strips	200
10	BAFRA, Gasa	100 strips			100
11	BAFRA, Haa	100 strips			100
12	BAFRA, Paro	100 strips			100
13	PAQS, Paro		100 strips	100 strips	200
14	BAFRA, Thimphu	100 strips	100 strips	100 strips	300
15	BAFRA, Tsirang	100 strips			100
16	BAFRA, Dagana	100 strips			100
17	BAFRA, Punakha	100 strips			100
18	BAFRA, Wangdue	100 strips			100
19	BAFRA, Phuentsholing	100 strips			100
20	PAQS, Phuentsholing		100 strips	100 strips	200
21	BAFRA, Samtse	100 strips			100
22	BAFRA, Gelephu	100 strips			100
23	PAQS, Gelephu		100 strips	100 strips	200
24	BAFRA, Zhemgang	100 strips			100
	Total	**2000 strips**	**500 strips**	**500 strips**	**3000**

ANNEX II – SAMPLING PLAN

SAMPLING PLAN A

Test using LF 680 - For corn/soyabean seed/leaf matrix - *used by all 20 Dzongkhags*

SAMPLE DETAILS

Sample type	*Number of samples*	*Recommended point of sampling*
Corn seed (imported)	*20*	*Retail shops selling corn*
Corn seed (local)	*20*	*Retail shops selling corn*
Soyabean seed (imported)	*20*	*Retail shops selling soyabean*
Soyabean seed (local)	*20*	*Retail shops selling soyabean*
Corn leaf	*10*	*From farms /nurseries growing corn*
Soyabean leaf	*10*	*From farms /nurseries growing corn*

SAMPLING METHOD: ***SYSTEMATIC RANDOM SAMPLING WAS USED***

SELECTION OF SHOPS FOR COLLECTING SEED SAMPLES

METHOD I *- For Dzongkhags with* ***MORE*** *than 20 number of shops in one town*

1. Find approximate number of shops selling local/imported corn and soyabean in the form of seed/grain to draw 40 samples of corn *(20 local and 20 imported)* and 40 samples of soyabean *(20 Local and 20 imported).*
2. Divide the total number of shops by 20 and this will give sampling interval for Systematic Random Sampling. *(For example, if there are 60 shops, then 60 /20=3. Then 3 is the sampling interval)*
3. Now pick a random number from 1 to 3 *(Write 1, 2, and 3 on a piece of paper, fold it and randomly pick one*). If you picked number 2, then Shop number 2 is the first shop for you to sample. For next sampling point, pick every 3rd shop. The Second Sampling point will be Shop no 5 (2+3), the subsequent sampling points will be 8th, 11th, 14th…….
   If the selected sampling point doesn't have the required sample, go to the next sampling point.

4. If you could not get 40 samples, then repeat the cycle from your first starting point.
5. From selected shops, sample both local and imported seeds *(Where possible)*

Note: Select shops Street wise and stick to the same side of the street. When you reach the end of the street, go to the other side of the street or the next street.

***METHOD II -** For Dzongkhags with **LESS** than 20 number of shops*

- Select the town with the maximum number of shops in the Dzongkhag.
- Select all the shops in that town, go to other towns if there is no sufficient number of shops.
- Draw total of 40 samples of corn *(20 Local and 20 imported)* and 40 samples of soyabean *(20 Local and 20 imported)* from different shops.
- From selected shops, sample both local and imported seeds *(Where possible)*

SAMPLING PROCEDURE FOR METHOD I and II:

- *Choose a bag/sack containing corn/soyabean seeds, mix it with your hands (using gloves), then **select one seed randomly**.*
- *For sample preparation and assay procedure, follow the video guide and the test protocol.*
- *Interpret the results as per the Interpretation Guide*

SAMPLING FRESH LEAVES SAMPLES

- *Randomly select 10 farms/nursery growing corn and 10 farms/nursery growing soyabean (where information is available, prioritize those households who have imported seeds or purchased from others)*
- *From each farm/nursery – **draw one leaf sample randomly**.*
- *For sample preparation and assay procedure, follow the video guide and the test protocol.*
- *Interpret the results as per the Interpretation Guide.*

SAMPLING PLAN B

TEST USING LF 681 and LF 682 - For corn/soybean bulk grain

(Used by all PAQS Samdrup Jongkhar/Paro/Phuentsholing/Gelephu and Thimphu offices)

SAMPLE DETAILS

Sample type	*Number of samples*	*Recommended point of sampling*
Corn seed (imported)	*25*	— *From feed processing companies* — *Bulk grain storage* — *From corn/soybean wholesaler*
Corn seed (local)	*25*	
Soya bean seed (imported)	*25*	
Soya bean seed (local)	*25*	

SAMPLING POINT SELECTION METHOD –A SIMPLE RANDOM SAMPLING TECHNIQUE WAS USED

1. Collect a list of **WHOLESALER/FEED COMPANIES** in your Dzongkhag
2. From the sampling frame list, select required number of sample collection points using simple random sampling technique
3. From selected shops/plants, sample both local and imported seeds *(Where possible)*

SAMPLING PROCEDURE:

1. *Select bags in the store/shops/companies where corn/soyabean are stored in bulk.*
2. *Randomly select sample from the bags as below:*
   - ***Corn = sample 1000 beans (approx 250gms) from each bag***
   - ***Soyabean = 1000 corn (approx 150gms) from each bag***
3. *For sample preparation and assay procedure, follow the video guide and the test protocol.*
4. *Interpret the results as per the Interpretation Guide.*

ANNEX III - Guide for assigning Sample ID

Sample ID = *Dzongkha Code-Serial no*

Eg: Sample ID for first sample in Trongsa is ***TR-001***

Dzongkhag Code is as provided below

S.N	*Name of Office*	*Dzongkha Code*
1.	*BAFRA, Trongsa*	*TR*
2.	*BAFRA, Bumthang*	*BT*
3.	*BAFRA, Mongar*	*MG*
4.	*BAFRA, Lhuntse*	*LT*
5.	*BAFRA, Tashigang*	*TG*
6.	*BAFRA, Tashiyangtse*	*TY*
7.	*BAFRA, Pemagatsel*	*PG*
8.	*BAFRA, Samdrupjongkhar*	*SJ*
9.	*PAQS, Samdrupjongkhar*	*ESJ*
10.	*BAFRA, Gasa*	*GS*
11.	*BAFRA, Haa*	*HA*
12.	*BAFRA, Paro*	*PR*
13.	*PAQS, Paro*	*EPR*
14.	*BAFRA, Thimphu*	*TP*
15.	*BAFRA, Tsirang*	*TS*
16.	*BAFRA, Dagana*	*DG*
17.	*BAFRA, Punakha*	*PN*
18.	*BAFRA, Wangdue*	*WD*
19.	*BAFRA, Phuentsholing*	*PL*
20.	*PAQS, Phuentsholing*	*EPL*
21.	*BAFRA, Samtse*	*ST*
22.	*PAQS, Samtse*	*EST*
23.	*BAFRA, Gelephu*	*GL*
24.	*PAQS, Gelephu*	*EGL*
25.	*BAFRA, Zhemgang*	*ZG*

ANNEX IV – TEST PROTOCOLS

Rapid lateral flow strip (LFS) for detection of CP4EPSPS (RR) in Corn/Soybean leaf or seed

Cat No. AID 025

AMAR
IMMUNODIAGNOSTICS PVT. LTD

Intended use

CP4EPSPS (RR) lateral flow strips (LFS) test kit is intended to be used for the qualitative detection of CP4EPSPS protein in individual Corn/Soybean leaf or seed samples.
The total incubation time of the assay is 5 minutes.


Punching a Corn leaf in a microfuge vial

Principle of the test:

An antibody specific to the CP4EPSPS protein molecule is immobilized on the test line area of nitrocellulose membrane. Second antibody specific for CP4EPSPS molecule is conjugated with colloidal gold and incorporated in the sample area of lateral flow strip. Anti mouse IgG is immobilized on the control line area of the LFS.

When the LFS is placed in the sample extract, the CP4EPSPS protein present in the sample extracts binds to the antibody labelled with gold and the complex moves upward by capillary action. The complex then binds to the antibody coated on the test line resulting in pink/purple color test line. As the complex moves further up, it binds to the control line resulting in pink/purple color control line. In absence of CP4EPSPS, the test line does not appear as no complex binds to the test line while control line turns pink/purple color indicating validity of test protocol.

Contents of the kit: Kit is sufficient for 100 tests.

CP4EPSPS LFS strips	50 strips per canister. Two canisters per kit
Extraction buffer	Two vials of 50 ml each.
Dropper	One
Pack insert	One

Material and equipment required but not provided

* Pipette with disposable plastic tips
* 1.5 ml Microfuge tube with cap
* Pestles
* Timer
* Marking pen and Paper towels.

Precautions

The CP4EPSPS LFS kit is intended for in vitro use only. The reagents contain thimersol as preservative. Prevent direct skin and eye contact with kit components. Seek medical attention in case of accidental ingestion of kit components.

Storage of the kit

The kit should be stored at 2 - 8° C. The unopened kit is stable till the expiry date printed on the kit label. The cap of the canister should be closed firmly after removing the required strips. Exposure to moisture is likely to affect the performance of the test strips.

Sample preparation:

Extraction of seed tissue: Crush single seed and transfer it to 5 ml plastic tube. Add 1.0 ml of extraction buffer. Mix well and wait for five minutes.

Or

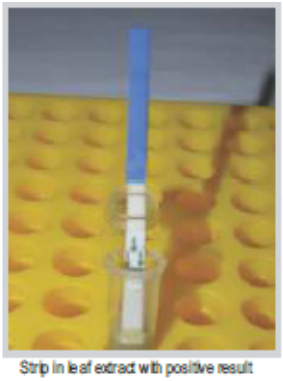
Use 48 well seed crushing plate, seed crusher and hammer to crush 48 seeds at a time. Add 1.0ml of extraction buffer using calibrated dropper. Mix well and wait for 10 minutes.

Extraction of leaf tissue:
Take two leaf punch (approximate weight 20 mg) and transfer it to 1.5 ml microfuge tube. Grind the leaf tissue with pestle until the leaf tissue is well ground. Add 0.5 ml extraction buffer. Mix well and wait for 5 minutes. Use new pestle for each leaf sample to avoid cross contamination.

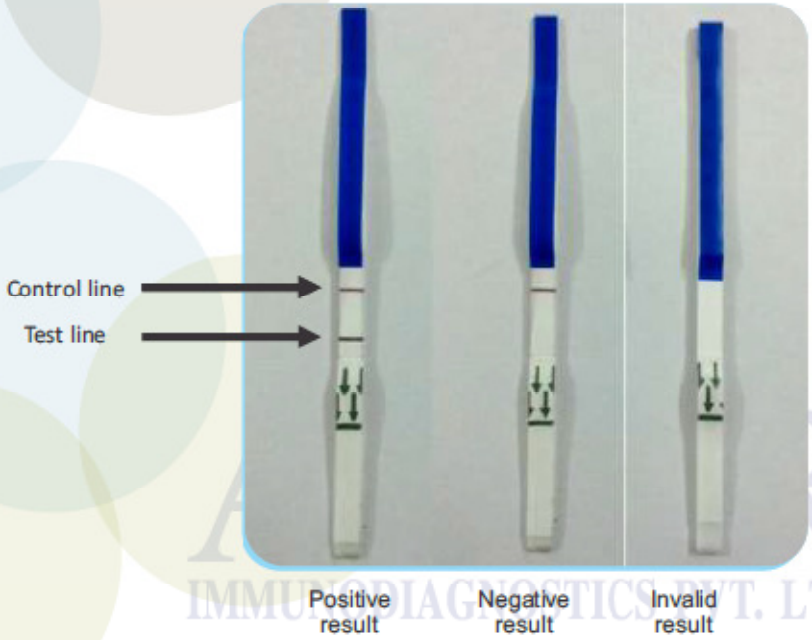

Extracting protein by crushing with pestle

Assay Procedure:

1. Allow canister to come to room temperature before opening it to remove the desired number of strips.
2. Insert one strip in each sample. Part of the strip showing arrow should be dipped in sample extract. Allow the test strip to remain in the seed crushing plate/microfuge/plastic tube in vertical position for 5 minutes.
3. Remove the strips and observe the results. Positive sample results may appear much earlier than 5 minutes.
4. For permanent storage of strips, cut off the bottom section of the strip covered with arrow using pair of scissors.



Strip in leaf extract with positive result



Cutting of strip for permanent record and storage

Interpretation of LFS results:

Read the strip in 5 minutes.

Two line indicates positive test result while single line indicates negative results.

Absence of control line in 5 minutes indicates invalid test.

The appearance of faint test line after 5 minutes should not be necessarily interpreted as positive test.

Rapid lateral flow strip (LFS) for detection of CP4EPSPS (RR) in Bulk grain Soybean

Cat No. AID 040

AMAR IMMUNODIAGNOSTICS PVT. LTD.

Intended use


Bulk Soy bean grain

CP4EPSPS (RR) lateral flow strips (LFS) test kit for bulk grain soybeans is intended to be used for the qualitative detection of CP4EPSPS protein present in Roundup Ready soybean. The kit detects one Roundup Ready soybean in 1000 conventional soybeans (0.1%).

The total incubation time of the assay is 10 minutes.

Principle of the test:

An antibody specific to the CP4EPSPS protein molecule is immobilized on the test line area of nitrocellulose membrane. Second antibody specific for CP4EPSPS molecule is conjugated with colloidal gold and incorporated in the sample area of lateral flow strip. Anti mouse IgG is immobilized on the control line area of the LFS.

When the LFS is placed in the sample extract, the CP4EPSPS protein present in the sample extracts binds to the antibody labelled with gold and the complex moves upward by capillary action. The complex then binds to the antibody coated on the test line resulting in pink/purple color test line. As the complex moves further up, it binds to the control line resulting in pink/purple color control line. In absence of CP4EPSPS, the test line does not appear as no complex binds to the test line while control line turns pink/purple color indicating validity of test protocol.

Contents of the kit: Kit is sufficient for 100 tests.

CP4EPSPS LFS strips	50 strips per canister. Two canisters per kit
Dropper	100
Microfuge tubes	100
Pack insert	One

Material and equipment required but not provided

* Waring blender	* Graduated cylinder	* Glass jars
* water	* Pair of scissors	* Timer

Precautions

The CP4EPSPS LFS kit is intended for in vitro use only. The reagents contain thimersol as preservative. Prevent direct skin and eye contact with kit components. Seek medical attention in case of accidental ingestion of kit components.

Storage of the kit

The kit should be stored at 2 - 8° C. The unopened kit is stable till the expiry date printed on the kit label. The cap of the canister should be closed firmly after removing the required strips. Exposure to moisture is likely to affect the performance of the test strips.

Sample preparation guideline :

Please use following link as a guideline to decide sampling strategy

http:/www.archive.gipsa.usda.gov/biotech/sample1.htm

Practical application of sampling for the detection of Biotech grains

To detect 0.1% Roundup Ready soybeans at 95 % confidence, it is necessary to have 3 sub samples of 1000 beans each and all three sub samples should test negative. Weight of 1000 beans is 150 gm.


Grinding into fine powder using a blender

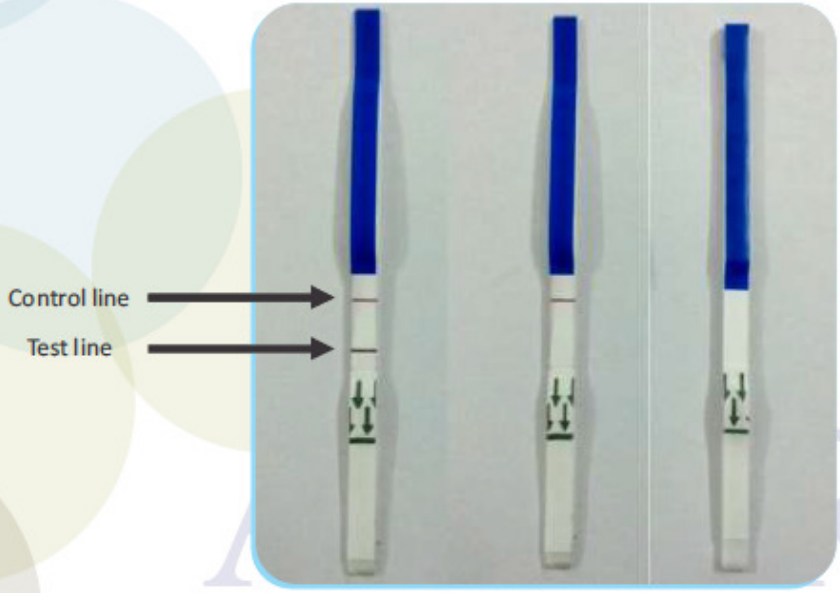
Sample preparation:

1. Weigh soybeans (Approximate weight of one bean is 0.15 gm) in to appropriate size jar.
2. Place cover on the jar and grind it in a blender on high speed for 45 seconds or till fine grain powder is observed.
3. Add required quantity of water to the jar (For 150 gm seeds, add 750 ml water)
4. Shake jar vigorously till entire sample is properly mixed. Allow sample to settle following which liquid from top can be collected. Draw 0.5 ml sample using calibrated dropper and transfer it to 1.5 ml microfuge tubes.

Assay Procedure:

1. Allow canister to come to room temperature before opening it to remove the desired number of strips.
2. Insert one strip in each sample. Part of the strip showing arrow should be dipped in sample extract. Allow the test strip to remain in the microfuge tube in vertical position for 10 minutes.
3. Remove the strip and observe the result. Positive sample result may appear much earlier than 10 minutes.
4. For permanent storage of strips, cut off the bottom section of the strip covered with arrow using pair of scissors.

Strip with positive result in bulk grain extract



Interpretation of LFS results:

Read the strip in 10 minutes.

Presence of control line in 10 minutes indicates that the strip has performed properly. The absence of control line in 10 minutes makes test invalid and should be repeated.

If the extract is from sample containing at least 0.1% RR soybean (one RR soybean in 1000 conventional beans) , the test line will appear and hence sample should be treated as positive.

The appearance of faint test line after 10 minutes should not be necessarily interpreted as positive test.

Cutting of strip for permanent record and storage

Rapid lateral flow strip (LFS) for detection of CP4EPSPS (RR) in Bulk grain corn

Cat No. AID 042

AMAR
IMMUNODIAGNOSTICS PVT. LTD.

Intended use

CP4EPSPS (RR) lateral flow strips (LFS) test kit for bulk grain corn is intended to be used for the qualitative detection of CP4EPSPS protein present in Roundup Ready corn. The kit detects one Roundup Ready corn in 1000 conventional corns (0.1%).

The total incubation time of the assay is 10 minutes.

Bulk grain Corn

Principle of the test:

An antibody specific to the CP4EPSPS protein molecule is immobilized on the test line area of nitrocellulose membrane. Second antibody specific for CP4EPSPS molecule is conjugated with colloidal gold and incorporated in the sample area of lateral flow strip. Anti mouse IgG is immobilized on the control line area of the LFS.
When the LFS is placed in the sample extract, the CP4EPSPS protein present in the sample extracts binds to the antibody labelled with gold and the complex moves upward by capillary action. The complex then binds to the antibody coated on the test line resulting in pink/purple color test line. As the complex moves further up, it binds to the control line resulting in pink/purple color control line. In absence of CP4EPSPS, the test line does not appear as no complex binds to the test line while control line turns pink/purple color indicating validity of test protocol.

Contents of the kit: Kit is sufficient for 100 tests.

CP4EPSPS LFS strips	50 strips per canister. Two canisters per kit
Dropper	100
Microfuge tubes	100
Pack insert	One

Material and equipment required but not provided

*Waring blender	*Graduated cylinder	*Glass jars
*water	*Pair of scissors	*Timer

Precautions

The CP4EPSPS LFS kit is intended for in vitro use only. The reagents contain sodium azide as preservative. Prevent direct skin and eye contact with kit components. Seek medical attention in case of accidental ingestion of kit components.

Storage of the kit

The kit should be stored at 2 - 8° C. The unopened kit is stable till the expiry date printed on the kit label. The cap of the canister should be closed firmly after removing the required strips. Exposure to moisture is likely to affect the performance of the test strips.

Sample preparation guideline :

Please use following link as a guideline to decide sampling strategy
http:/www.archive.gipsa.usda.gov/biotech/sample1.htm
Practical application of sampling for the detection of Biotech grains

To detect 0.1% Roundup Ready corn at 95 % confidence, it is necessary to have 3 sub samples of 1000 corn seeds each and all three sub samples should test negative. Weight of 1000 corn seeds is 250 gm.

Grinding into fine powder using a blender

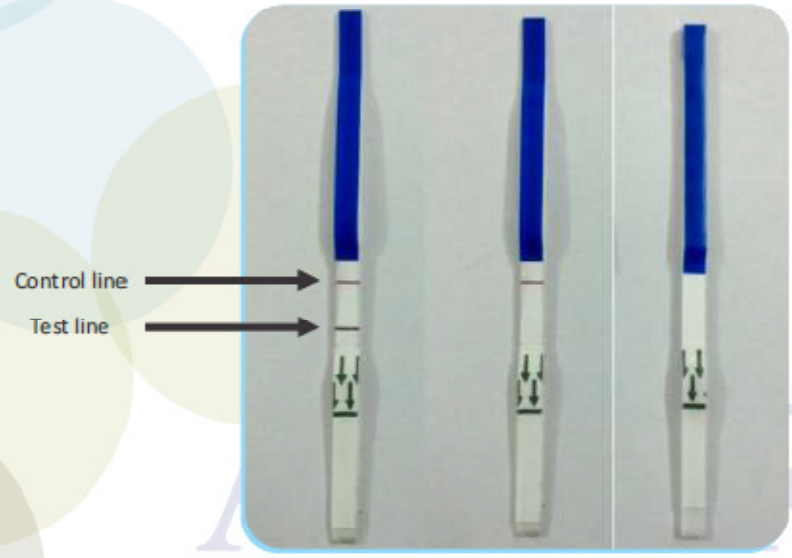
Sample preparation:

1. Weigh corn seeds (Approximate weight of one corn seed is 0.25 gm) in to appropriate size jar.
2. Place cover on the jar and grind it in a blender on high speed for 45 seconds or till fine grain powder is observed.
3. Add required quantity of water to the jar (For 250 gm seeds, add 380 ml water)
4. Shake jar vigorously till entire sample is properly mixed. Allow sample to settle following which liquid from top can be collected. Draw 0.5 ml sample using calibrated dropper and transfer it to 1.5 ml microfuge tubes.

Assay Procedure:

1. Allow canister to come to room temperature before opening it to remove the desired number of strips.
2. Insert one strip in each sample. Part of the strip showing arrow should be dipped in sample extract. Allow the test strip to remain in the microfuge tube in vertical position for 10 minutes.
3. Remove the strip and observe the result. Positive sample result may appear much earlier than 10 minutes.
4. For permanent storage of strips, cut off the bottom section of the strip covered with arrow using pair of scissors.

Strip with positive result in bulk grain extract



Interpretation of LFS results:

Read the strip in 10 minutes.

Presence of control line in 10 minutes indicates that the strip has performed properly. The absence of control line in 10 minutes makes test invalid and should be repeated.

If the extract is from sample containing at least 0.1% RR Corn (one RR Corn in 1000 conventional Corn), the test line will appear and hence sample should be treated as positive.

The appearance of faint test line after 10 minutes should not be necessarily interpreted as positive test.

Cutting of strip for permanent record and storage

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