

# Sampling protocol for MLN and Introduction to Immunostrips for MLN pathogen detection

*Workshop on Survey and Sampling for MLN Pathogens; April 8-9, 2017; CIMMYT, Kenya*

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# Overview

- Field sampling
- Sample processing
- Sample testing
- Documentation
- Sample shipping for ELISA testing.

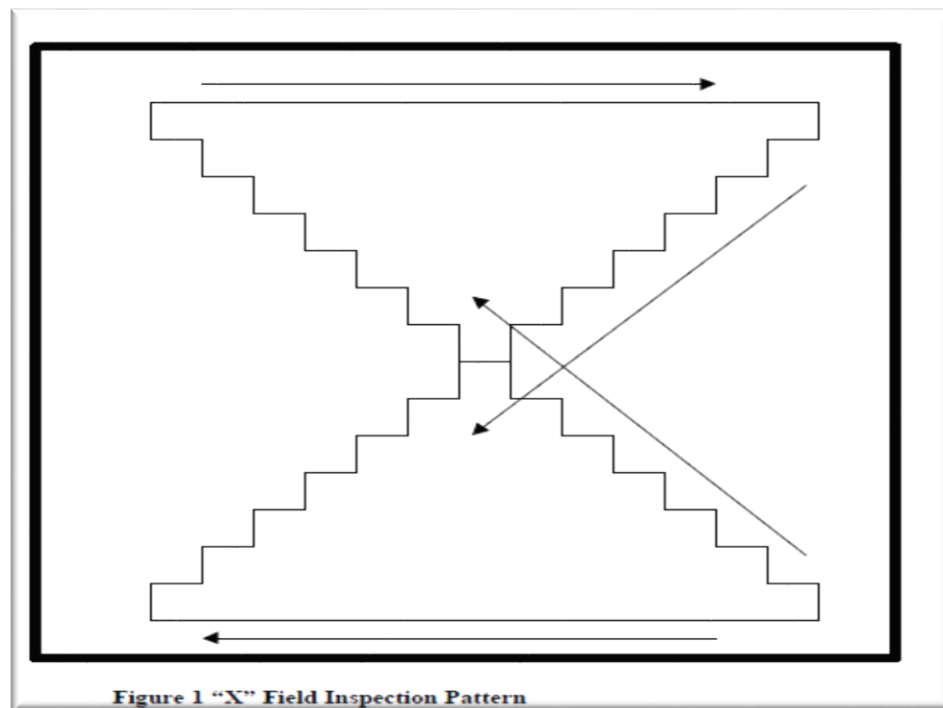


**How to collect and ship  
Fresh samples  
for the **VIRUS** diagnosis**

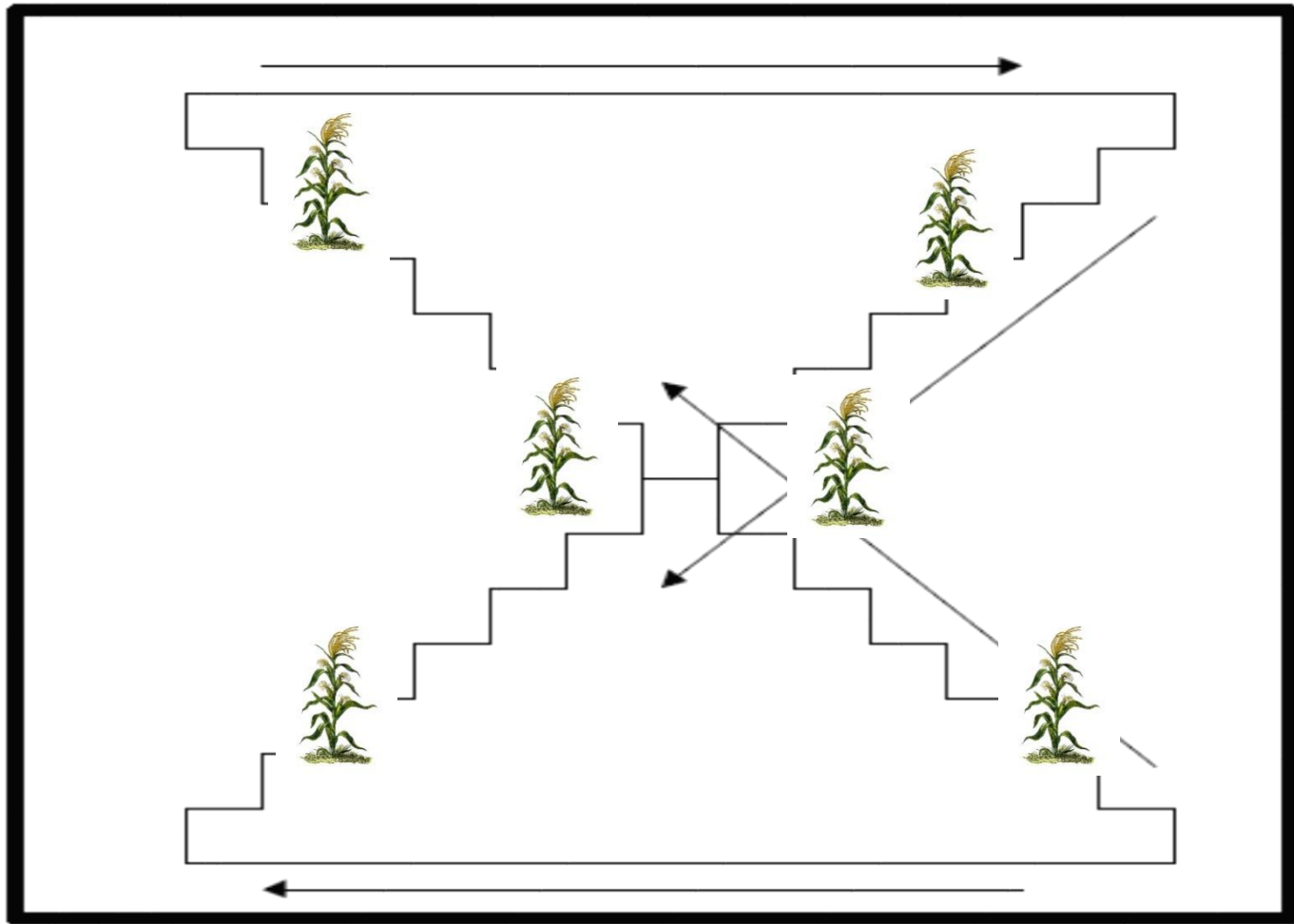


## FIELD INSPECTION and LEAF SAMPLING:

- The sampling procedure must ensure that all parts of the field are adequately and proportionately represented in the plants inspected with in the various usual microclimates of the field.
- The pattern of field inspection can vary as far as the above condition is met



(CDFA Phytosanitary Certification Manual, 1985)



**Figure 1 "X" Field Inspection Pattern**

# Procedure for leaf sampling

## Precautions :

- Choose the **right plant and leaf**.
- **Avoid** the plants with **abiotic symptoms** such as (mechanical ) wilt, nutrition deficiency, pesticide injury, due to drought.
- Try to **avoid samples with multiple symptoms** such as leaf blight, yellowing along with mosaic.
- If you are in doubt, please look for fresh plant sample.
- It is important that the samples should be collected before pesticide application.



# Selection of plant and collection of leaf tissue

- The sample should be taken from the youngest leaf of the plant. To carry out the sampling procedure, the diagnostician should wear laboratory gloves.
- Leaf samples should be collected as follows:
  - Select approximately a leaf of **newly developed symptomatic tissue**.  
Using a clean sheet of fresh tissue paper, enclose and hold the leaf to be sampled. Use bleach-cleaned scissors to cut off a 5-6 cm leaf segment (Fig 2). Carefully avoid cross-contamination of samples with exudate from leaves onto hands or implements. Scissors or any other implements must be cleaned thoroughly with bleach solution between each sample ( between farm).



2. Samples with no symptoms (a) and symptoms (b)

## Cover the leaf tissue and place it in perforated paper bag:

Fold the tissue paper so that it covers the leaf sample. Place the single collected leaf sample into a paper bag perforated with air holes (Fig 3) [NB: Only 1 leaf sample should be placed into each leaf sample bag]. Caution must be used not to touch the interior of the sample bag with fingers, implements or any other leaves.

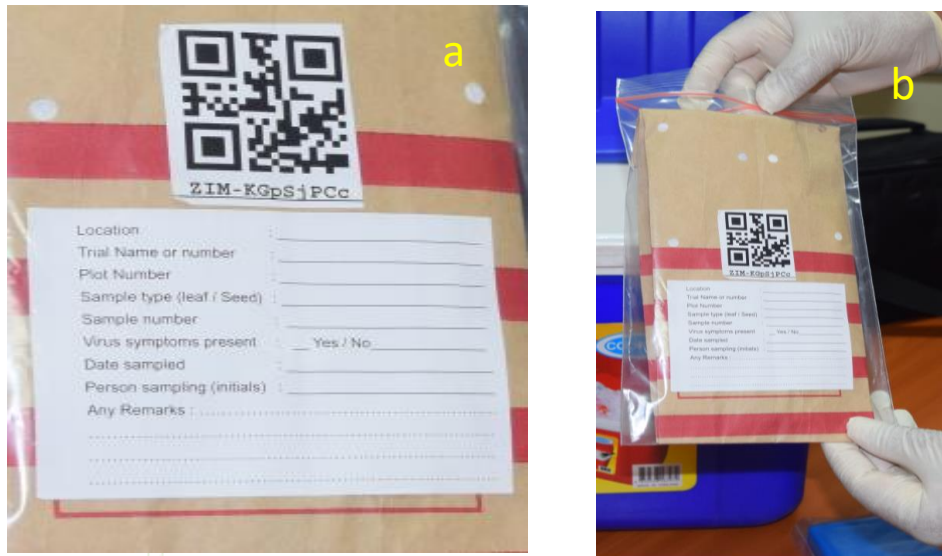


3. Placing the samples Between the layers of Tissue paper (a) and Inserting the samples In the perforated paper bag (b).



# Labelling and packing

- Stick completed sample labels and a unique QR code on each sample bag and record the unique sample code. Place each labelled sample bag inside a zip lock plastic bag, this will protect the label from damage).



**Fig 4. A. Individual leaf sample bag labeled with QR code and sample label. B. Labeled leaf sample bag inside ziplock bag**

# Sample bulking

- Place 6 individually labelled leaf sample bags from the same plot inside one large plastic bag (Bulk sample bag). This will keep the 6 leaf samples from the plot to be used for one bulk immunostrip assay together (Fig. 5). It is essential to stick another unique QR label onto this bulk sample bag and record the unique bulk sample code. Always use separate bags for each set of 6 leaf samples i.e., if 12 leaf samples in total are collected from a plot there should be 2 uniquely labeled bulk sample bags for the plot.

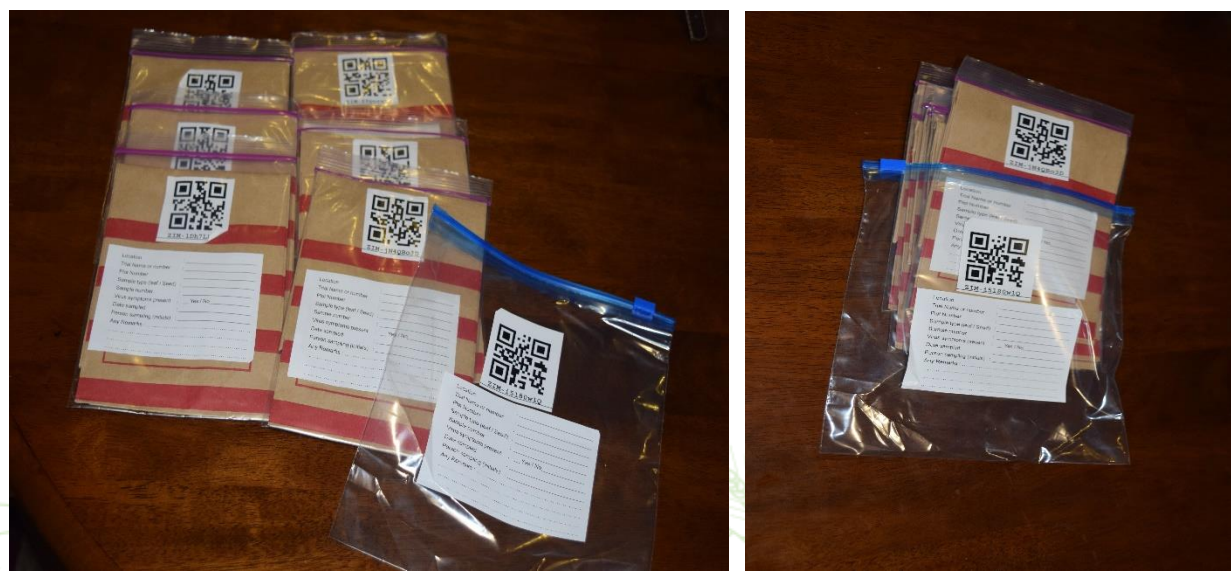


Fig. 5. Labelled bulk sample bag containing 6 individual labelled leaf sample bags



# Storing the samples in the container

Place the labeled bulk sample bags inside a cool box, and ensure that ice packets are placed around the samples to keep them cool during sample processing and subsequent transportation.



- ❖ Keep samples in a cool place until shipping. (Do not place in the refrigerator).
- ❖ When ready to ship, place the double bagged box containing in a styrofoam container with a coolpack. Place the styrofoam container in a cardboard box and ship as one unit.
- ❖ Label the outside of the box (Styrofoam) with sample ID, date sample was collected, grower and field where sample was collected. ( place a sample log details inside the box)
- ❖ Fill shipping container with packing material to avoid movement of the sample in the container

Fig. 6. Keeping the samples inside the ice box using ice pack



# FIELD AND LABORATORY DETECTION of MCMV:

## Immunostrips and ELISA

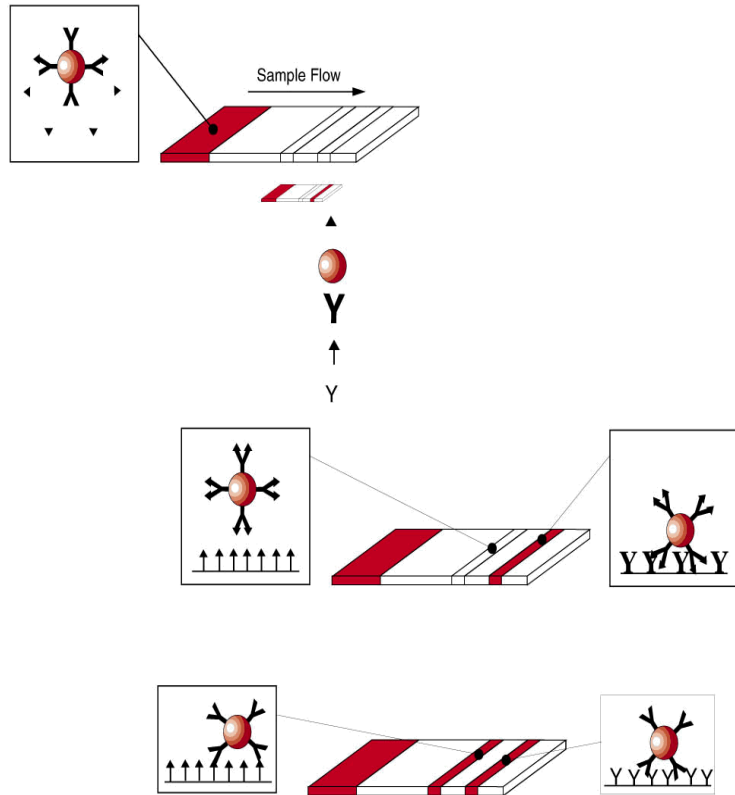
Both tests are based on the unique nature of the way in which the antigen (example: a plant virus) and an antibody molecule fit together

## FIELD AND LABORATORY DETECTION of MCMV:



# Fast detection with immunostrips:

## Principle:



- The technology is based on a series of capillary beds, such as pieces of porous paper, that has the capacity to transport fluid (e.g., plant sap) spontaneously.
- The porous paper acts as a sponge, once soaked, the plant sap migrates to the second section of the porous paper in which conjugate is stored.
- The analyte (plant sap containing the virus) binds to the particles while migrating further through the third capillary bed.
- This material has one or more areas where a third molecule has been immobilized by the manufacturer. By the time the sample-conjugate mix reaches these strips, analyte has been bound on the particle and the third 'capture' molecule binds the complex.
- After a while, when more and more fluid has passed the stripes, particles accumulate and the stripe-area changes color.

## Typically there are at least two stripes:

- one (the control) that captures any particle and thereby shows that reaction conditions and technology worked fine,
- the second contains a specific capture molecule and only captures those particles onto which an analyte molecule has been immobilized.

The benefits of immunochromatographic, also called lateral flow tests or simply strip tests, include:

1. User-friendly format.
2. Very short time to get test result.
3. Long-term stability over a wide range of climates.
4. Relatively inexpensive to make.



# Immunostrip test steps

- Selection of test place
- Keep at most hygiene as possible
- Carefully handle the leaf sample using glove
- Keep all the items sanitized
- Keep all the material check list ready
- Ensure the test documentation is done before you leave the place
- If needed or in doubt please contact the nearest contact or send mail to : [l.m.suresh@cigar.org](mailto:l.m.suresh@cigar.org) or whats up +254392664

- Avoid cross contamination between the bulk samples
- Avoid taking food, using phone, smoking etc..
- After the test is done, leave the sample in the same farm



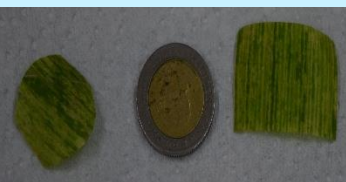
## Preparation of the sample:

1. For the correct execution of the test the ratio between leaf tissue and extraction buffer must be as close as possible to 1:40 w/v (or the one indicated in the instructions of the kit)
2. To maintain this proportion the total amount of tissue to be processed in the test must not exceed the size of a coin
6. Wearing gloves, open the first collection bag and detach a small portion of the tissue, introduce it in the plastic extraction bag
7. Close the collection bag where the samples was preserved and store the bag again in the cool box
8. Change gloves between samples
9. Wear a new pair of gloves and proceed as in point 6 until you obtain the 6 leaf fragments to test in the extraction bag
10. Add the buffer as indicated on the instructions included with in the kit
11. Homogenize the sample with .....on a flat surface; for this purpose a plastic box or a plastic tray can be used as working bench. This action should not be longer than 2-5 seconds
12. Transfer a total of 4 drops of the extract into the disposable cuvette
13. Insert 1 strip as indicated on the instructions included with the kit
14. Leave the strip for at least 10-15 minuets before reading the results
15. Interpretation of the results:



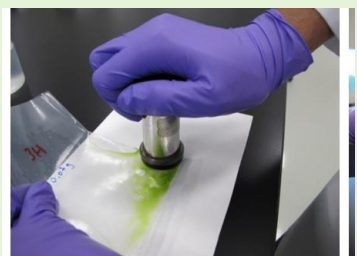
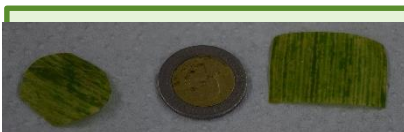
# Immunostrip test steps

Sample collection



5 min

Sample Preparation



5 min

Immunostrp test

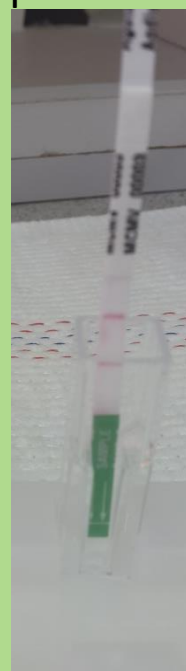


1 drop

3 drop

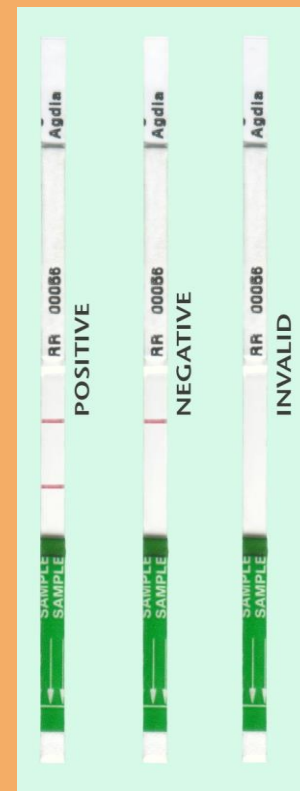
sap

buffer



15 min



Documentation



5 min

MYT<sub>MR</sub>

# MCMV immunostrip documentation

| MCMV Diagnostic Testing of Maize Plant Samples |   |                                      |   |          |
|--|---|--------------------------------------|---|----------|
| Location :                                     | Harare  |                                      |   |          |
| Farmer's Name :                                | Jan De Vries  |                                      |   |          |
| Trial Name or Number (if applicable):          |   |                                      |   |          |
| Plot Number (if applicable):                   |   |                                      |   |          |
| Virus symptoms present: Yes / No               | Yes   |                                      |   |          |
| Date of sample :                               | 3 <sup>rd</sup> March 2014  |                                      |   |          |
| Sample location :                              | Harare  |                                      |   |          |
| Name of the person who sampled:                | Kevin Conn  |                                      |   |          |
| Bulk Sample ID                                 | Immunostrip (paste the strip here)  | Reaction start time (in min and sec) | Reaction confirmation time (in min and sec) | Remarks  |
| ZWEwoodfrtds12                                 |    | 2.13                                 | 4.13  | Positive |
| Signature :                                    |   |                                      |   |          |
| Name :   | Kevin Conn  |                                      |   |          |
| Institution :                                  | ZARI  |                                      |   |          |
| Location :                                     | Harare  |                                      |   |          |
| Farmer's Name :                                | Jan De Vries  |                                      |   |          |
| Trial Name or Number (if applicable):          |   |                                      |   |          |
| Plot Number (if applicable):                   |   |                                      |   |          |
| Virus symptoms present: Yes / No               | Yes   |                                      |   |          |
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| Signature :                                    |   |                                      |   |          |
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| Institution :                                  | ZARI  |                                      |   |          |

**Disclaimer:**  
CIMMYT does not specifically endorse any specific commercial product / brand / company mentioned in this experiment.

## ❖ Sending Samples

1. *If MLN symptomatic plants (or suspected MLN symptomatic plants) are observed on the survey (i.e., any bulk sample that test positive with the immunostrip test), the samples should be sent to a recommended laboratory for ELISA testing for confirmation as soon as possible (within 48 hours).*
2. *If possible, mail the positive / suspected samples to a recommended laboratory in the country [NB: **NO samples should be mailed to another country**]. Place the bulk sample bag(s) inside a small cardboard box. Please mention on the box “**Plant sample**” “**RUSH IMMEDIATELY**” and mail using either a courier service or express mail to the laboratory.*
3. *If the samples cannot be mailed immediately, keep them in a refrigerated condition or in a cool dark place. Samples must be refrigerated at 4°C and kept for no longer than 48 hours. After that time, leaf samples can deteriorate and the results will not be reliable.*

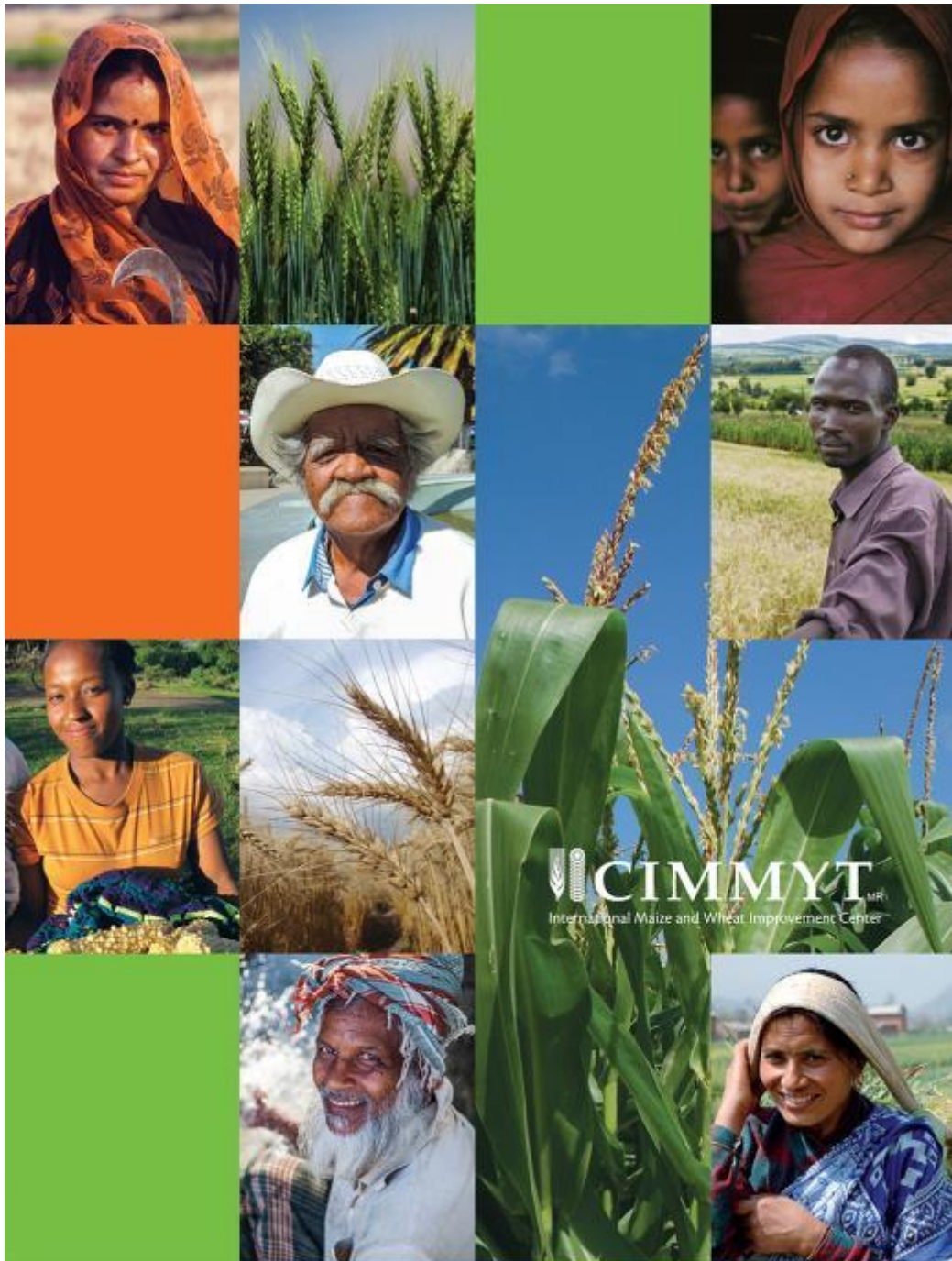


# Packing and shipping samples :

- After placing the bulk sample bags into the cool box take the samples to a suitable site / laboratory in the plot to undertake the ELISA
  - After completing the immunostrip tests and recording all sample information, place the Styrofoam / cool box with all the collected plot samples inside a cardboard box, to provide a good and sturdy transport arrangement (Fig. 9).
- **Note:** Inside each box, please keep a list of samples that has all the information collected during the sampling .



Fig. 9. Sample box ready for transportation



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for your  
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