



## 1. PURPOSE

The purpose of this standard operating procedure (SOP) is to determine the morphological composition on a dry matter basis, of Leaf-Stem-Dead components of tiller samples.

The grass plant is comprised of leaf, pseudostem, true stem (including inflorescence) and dead (Beecher et al., 2013). These components differ in digestibility and variations in their relative proportions can impact sward quality (Beecher et al., 2013). For accurate descriptions of a sward in grazing studies and to accurately determine sward morphological composition, pseudostem should be separated from true stem, particularly during the reproductive stage when true stem is present (Beecher et al., 2013).

Several morphological components make up the grass plant and these vary in digestibility (Stakelum and Dillon, 2007; Beecher et al., 2013). The leaf is comprised of the leaf blade (leaf) and leaf sheath; the collection of leaf sheaths on a tiller make up the pseudostem. During the reproductive stage true stem emerges upwards from the base of the tiller through the pseudostem (Beecher et al., 2013).

## 2. SCOPE

- This SOP is applicable to fresh herbage only.
- This SOP is applicable to all personnel determining the LSD components in the Grassland Laboratory.

## 3. DEFINITIONS/ABBREVIATIONS

- SOP - Standard Operating Procedure
- LSD - Leaf-Stem-Dead
- DM - Dry matter
- PPE – Personal Protective Equipment

## 4. EQUIPMENT AND MATERIALS

### Equipment

- Denver TP3002 top loading balance.
- Elastic bands
- Knife/Scalpel [Refer to sharps policy for further information and safety measures].
- Labelled plastic bag [Trial Code, Date, Paddock Number and Sample Number].
- Graduated ruler
- 4° C cold room
- 141x116x40mm foil trays
- 90° C drying oven
- Pre-Printed Tickets (stored in the 'Ticket Folder' in the Grassland Laboratory oven room), indicating the Trial Code, sample number and oven temperature.

## Materials

- Herbage tiller sample

## 5. PROCEDURE

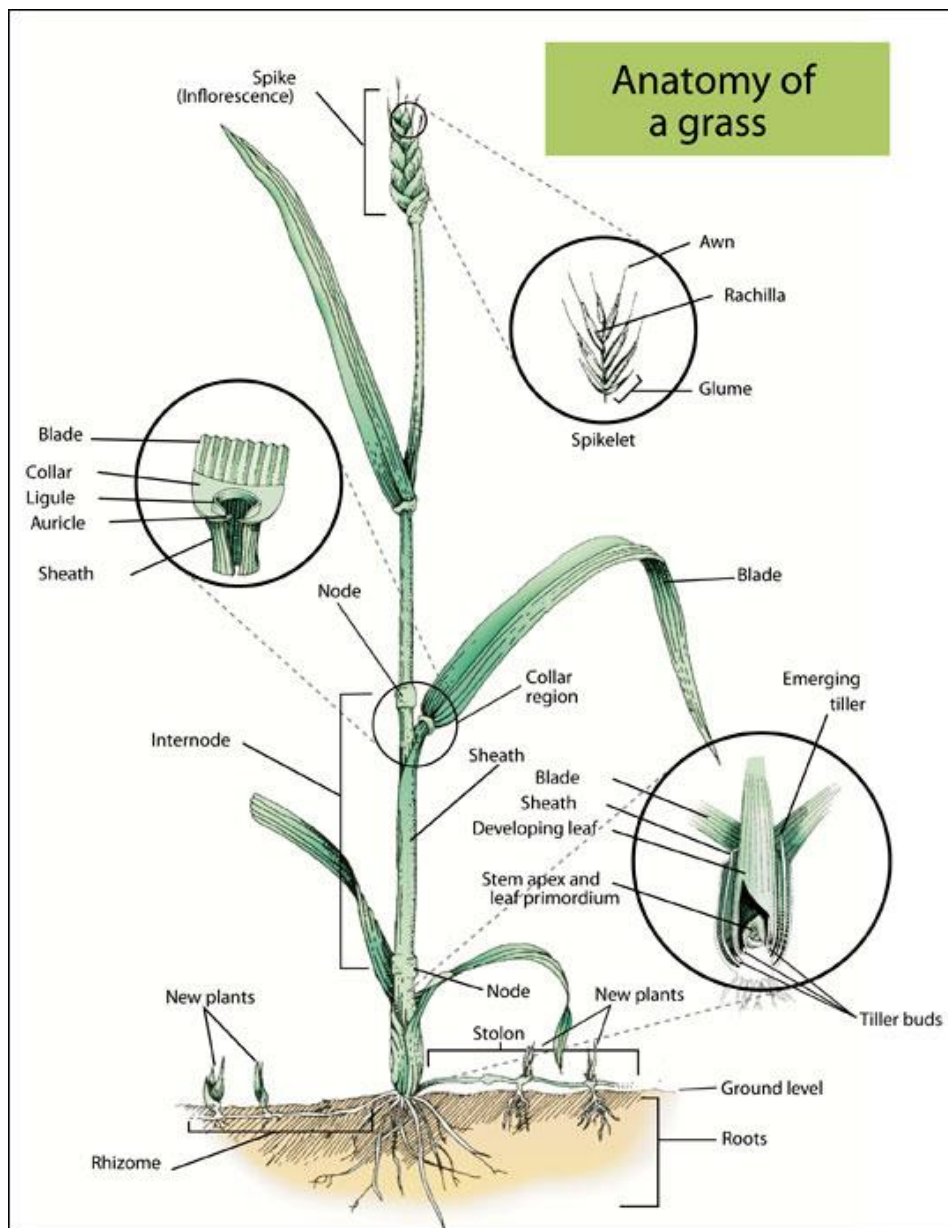
- **Caution:** Using a knife/scissors carefully cut approximately 100g of grass to ground level, from areas randomly selected across the paddock or plot [Refer to relevant SOP on tiller collection for more information].
- Secure the sample with an elastic band and place in a labelled plastic bag; so as to preserve the vertical structure of the sample.
- When the sample enters the laboratory it is immediately placed in the 4°C cold room (with a cold room label detailing the Trial Code and Oven Temperature), laid out on paper towel, in open plastic bags, to absorb any surface moisture and avoid decay, until ready for processing.
- All samples should be separated within 72 h of collection.
- When the sample is ready for processing, remove from the cold room, and from each sample, weigh out **40 grams (alternatively if the trial SOP's require- count out a pre-determined number of tillers)**, onto a top loading balance (previously tared to zero with a steel tray).
- If not required for subsequent analysis, such as determining the 'Extended tiller height, Extended sheath height and Free leaf lamina', dispose of the 60g unused sample moiety into the dungstead [Refer to the relevant laboratory waste SOP for greater detail].
- **Caution:** Using a graduated ruler, chopping board and knife/scalpel, cut the sample at **4cm or 3.5cm – the cut height depends on the trial specified requirements** [Refer to sharps policy for further information and safety measures].



- Discard to the dungstead, the portion below 4cm or 3.5cm.
- **Caution:** Using the knife/scalpel separate the above 4cm or 3.5cm portion into leaf, true-stem, pseudo-stem, and dead material, and place in individual pre-ticketed foil trays (see Figure 1 below).



- Dry each component for a minimum of 15hours at 90° C to determine morphological composition on a DM basis (see Figure 1 below). NOTE: as see from Figure 1 below, the foil trays are placed into larger steel trays so as to prevent them being knocked over in the oven.
- Using relevant PPE remove from oven and allow air to equilibrate for 15mins.
- Weigh the sample and record into appropriate database.
- Dispose of the dried laboratory sample moiety in the dungstead.



[www.1](#) Anatomy of a single grass tiller

**LEAF:** Each leaf has an upper part called the blade and a lower part called the sheath.

- Leaf blades to be detached from the base of the pseudostem or true stem- as classified as leaf.

**PSEUDOSTEM:** Is a false stem, comprised of rolled out leaf sheaths

- Leaf sheaths to be separated from the true stem and included in the pseudostem fraction.

**TRUESTEM:** Below the pseudostem is a true stem.

- The true stem has divisions called nodes.
- Inflorescences (seed head), if present, to be included as true stem.





Title: Determination of morphological composition on a dry matter  
basis of Leaf –Stem-Dead tiller samples

SOP-GL-028

**DEAD:** Dead matter defined as any senesced material that is yellow/brown in colour.





**Figure1: LSD samples placed within the 90° C**

## **6. RESPONSIBILITY**

- It is the responsibility of all personnel to assure that this SOP is performed as described.
- It is the responsibility of the research officer to ensure appropriate training and instruction is given before this SOP is undertaken.
- It is the responsibility of all personnel to report any problems that may occur while performing this procedure to their supervisor and Laboratory manager.

## **7. ENVIRONMENTAL HEALTH AND SAFETY:**

- Exert caution when using the oven.
- Please refer to Teagasc Laboratory Safety Manual for additional information.
- Please refer to Teagasc Bio-Safety Manual for additional information.
- Please refer to Laboratory Sharps policy for additional information.

## **8. ASSOCIATED RISKS**

- Slip/trip/fall
- Lacerations and cuts
- Burns
- Electric shock



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SOP-GL-028

## 9. **RECOMMENDED PPE**

- Heat resistant gloves
- Laboratory coat
- Gloves

## 10. **HELPFUL LINKS AND PHONE NUMBERS**

- Grassland Laboratory Manager: Michelle Liddane: [michelle.liddane@teagasc.ie](mailto:michelle.liddane@teagasc.ie)
- [SOP\\_GL\\_3\\_Lab\\_waste\\_Grassland\\_Laboratory.doc](#)
- [SOP\\_GL\\_6\\_Weighing\\_Scales\\_in\\_Field\\_and\\_Laboratory.doc](#)
- Beecher, M., Hennessy, D., Boland, T., McEvoy, M., O'Donovan, M., and Lewis, E., The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility

<http://t-stor.teagasc.ie/bitstream/11019/587/1/cultivar%20morphology%20paper%20d12%20post%20print.pdf>

- *Stakelum G. and Dillon P. (2007) The effect of grazing pressure on rotationally grazed pastures in spring/early summer on subsequent sward characteristics. Irish Journal of Agricultural and Food Research, 46, 15–28.*
- [www. 1 http://extension.missouri.edu/publications/DisplayPrinterFriendlyPub.aspx?P=M182](http://extension.missouri.edu/publications/DisplayPrinterFriendlyPub.aspx?P=M182)

Please contact Researcher in charge if you have any queries regarding this lab procedure

**For further information please consult the manual of any equipment required, the SDS for the chemicals involved and any relevant SOP's!**