- Different plant families may have embryos that require a specialized evaluation criterion. For example, unstained radicle tips are generally interpreted differently in grasses than in legumes.

- Abnormal staining patterns and TZ hue may be symptomatic of thermal or mechanical damage. Seed may be damaged by excessive heat or cold, or improper handling in the field, conditioning plant, or warehouse. Examples are: early freeze when seed has high moisture levels, overheating in windrows, mechanical damage during cleaning, improper storage conditions or damage during transport. Such damage may be evident in a TZ test as a watery red color, purplish red, or especially dark red and flaccid tissue.

- Some types of abnormal seedlings will not be detected in a TZ test. For example, seedlings may develop stunted roots in a germination test, even though radicles of embryos of seeds from the same sample stain normally in TZ. For this reason, paired TZ and germination tests should be conducted whenever possible.

15.1.3.1 False positives -

If seeds are over-stained, a false positive evaluation may result. This often occurs within the Poaceae family. Other tissues or other organisms (e.g. fungi or bacteria) can settle onto the cut surface of the seed and create a normal red staining appearance. With some seeds, the embryo and endosperm must both stain (eg. carrots and onions). If the embryo stains, but the endosperm does not (or vice versa), the seed may have low vigor, produce an abnormal seedling, or be dormant. Interpretation of the staining pattern of both the embryo and nutritive tissue is critical.

15.1.3.2 False negatives -

Seeds that have deep dormancy may not stain at all because of low respiration levels. The seed will only stain in certain stages of physiological development. The analyst must be aware of dormancy within species, ways to overcome or break dormancy, and how to interpret a ‘no stain’ or ‘light stain’ in this situation.

Artifact damage may also give a false negative evaluation. Keep cutting implements sharp to avoid this problem. This damage often shows up as a white coloration over the cut surface of the seed and masks the red coloration below. This frequently occurs on seed that have a hard, brittle seed coat or floral parts. Artifact damage may also manifest itself as dark red to black coloration on the cut surface of the seed. This is like a ‘bruise’ and occurs on seed with softer tissues.

Embryos with chlorophyll can be difficult to interpret. The presence of chlorophyll is not alone an indicator of viability. If the green chlorophyll is metabolically active, there is no red coloration (e.g. *Acer*). If the tissue texture is firm (turgid), these seeds are considered viable even though there is no stain. In many legumes (Fabaceae), the green embryos indicate immature seeds. The texture of the immature seeds is usually soft (flaccid), and the cotyledons do not expand as they imbibe. These seeds are considered non-viable. In some species, both the green coloration of the chlorophyll and the red of the TZ stain may be present, giving a brownish color or tint (e.g. *Phlox*, *Kochia*). If the embryo tissue is turgid and cotyledons are expanding, these are considered viable. Because of the difficulty in interpreting the TZ stain in seeds with green coloration from chlorophyll, a germination test is recommended.

15.1.3.3 Multiple seed units and multiple embryos -

Some families have species with multiple seed units, such as: beets, dogwood, little burnet, and yellow poplar. The issue of how to determine the percentage of viable seed can be confusing when evaluating these species. Each unit may have one or more viable embryos in the multiple seed unit. The reported results may need to clarify the number of seed units evaluated, the number of viable seeds, and the number of seeds with two or more viable embryos. This way, customers can determine the actual number of 'total germinants' that they will have in the seed lot. (This is especially important for bare-root nursery production.)

It is also possible to have multiple embryos in bluegrass, New Zealand spinach, and pine. With these species, the presence of at least one red-stained embryo is required to evaluate the seed as viable.

15.1.3.4 Immature seed

Immature seeds lack sufficient differentiation and/or development to germinate and produce a plant. Flaccid green embryos may be immature. Immature seeds may appear shrunken or smaller than other seeds in the sample. Essential structures are not differentiated and are not seen even with magnification. In the grasses, for example, the endosperm may be shrunken and the embryo may be a solid mass of stained tissue lacking differentiation into a scutellum and plumule/radicle axis. Such seeds are non-viable.

15.2 TZ as a vigor test

A vigor test is an indicator of how well the seed may perform under a wide range of field conditions. Vigor is associated with seed age, hardiness, soundness, and health and is a measure of the physiological condition of the seed. Vigor tests can include growth measurements, stress tests, and biochemical tests.

The tetrazolium test is a 'biochemical vigor test'. At the same time the viability is being evaluated, the TZ test can be used secondarily as a vigor test. The intensity of the staining reaction is used as a basis of seed vigor. Older seeds tend to stain a much darker red, take up little or no stain, have a mottled staining pattern, or have less tissue turgor. More vigorous seeds tend to stain pink to red and show turgid, firm tissue.

Four categories used to estimate vigor as the seed is being evaluated for germination:

1) high vigor 2) medium vigor 3) low vigor 4) non-germinable

Seeds are placed in the respective categories based on: intensity of staining, location of deteriorated and/or dead tissue, amount of dead or dying tissue, and the development of the embryo. For details, refer to the AOSA *Seed Vigor Testing Handbook, Contribution No.32* or the ISTA *Handbook of Vigor Test Methods.*