that were insufficiently scarified with acid will have to be treated again or mechanically scarified.

9. TEMPERATURE

Temperatures between 20-40° C (68-106° F) have no adverse effect on tetrazolium test accuracy. Staining rate increases as temperature increases. Tests may be performed satisfactorily at room temperature, but will take longer to stain. If shorter test periods are desired, heat can be supplied by placing tests in a 30-40° C oven or germinator. Do not use temperatures greater than 40° C. The general rule of thumb is that staining will take place twice as fast at 30° C than at 25° C, and twice as fast at 35° C than at 30° C. (see section 7.6)

10. LIGHT

Tetrazolium tests may be placed in subdued light or in darkness during the staining period. TZ exposed to light will turn from a clear or light yellow to a pink or reddish solution, but this will have no effect on the tetrazolium reaction within the seed.

11. PRESSURE (Partial Vacuum)

Osmotic potential present within the seed, seed chemistry (protein, lipid, or carbohydrate), tissue density and seed coat composition can affect tetrazolium infusion into vital seed tissues. Hard seed should be scarified so that tissues can hydrate and take up the TZ solution. Smaller seed with a soft caryopsis should be pierced, rather than cut, to avoid mechanical damage to the embryo. Placing pierced seed into a heated (30-45° C) vacuum oven chamber (to approximately 150 mm Hg) for one half to one hour will decrease the staining reaction time considerably. When using a vacuum oven, the TZ staining reaction time can generally be halved on seeds that require piercing.

12. SOURCE OF SEEDS TO TEST

Careful sampling procedures must be followed in obtaining seeds for testing if the results are to be representative of the viability of the entire seed lot. Since variability always exists in seed lots, composite samples should be obtained by mixing together samples taken from evenly distributed parts of the lot to be sampled. For free-flowing seed in bags or bulk, a probe, trier, or automatic sampler should be used. For non-free-flowing seed, representative portions may be taken by hand.

Detailed instructions on sampling for purity and germination tests, given in Section 1 of the *AOSA Rules for Testing Seeds Volume 1. Principles and Procedures,* are equally applicable for tetrazolium testing. As with the AOSA germination testing rules, only 'pure seed' is used in the TZ test. For 'pure seed' definitions, consult Section 3 of the *AOSA Rules for Testing Seeds Volume 1. Principles and Procedures*. For some species, 'pure seed' may include empty seeds. When testing those species, empty seeds discovered in the TZ should be counted as non-viable.

13. NUMBER OF SEEDS TO TEST

Sampling error in the TZ test is the same as it is for germination tests. However, because of the greatly increased analyst bench time, the need for expediency has resulted in a compromise regarding the number of seeds tested. For stand-alone TZ tests most are conducted with a minimum of 200 seeds in replicates of 100 seeds or fewer. In a few states where the tetrazolium test is allowed as a primary viability indicator, a 400 seed test is required. The seeds should be randomly selected and counted out in replicates before any tetrazolium testing procedure begins. Often a few additional seeds are counted out at the beginning of the test to allow for analyst error or experimentation in seed preparation (cutting or piercing). When less precision is required and when seeds are extremely difficult to prepare for staining, seed tests of fewer than 200 seeds may be warranted.

With a mixture, 200 seeds of the majority pure seed component and 100 seeds of the minority pure seed component should be tested. Requirements vary among states.

When testing a seed lot for dormancy at the end of the germination test period, the tetrazolium test is performed on firm, ungerminated seeds. The TZ test might also be paired with a germination test from the same seed lot to account for a potential difference related to dormancy. (see section 6)

14. PREPARATION OF SEED FOR TESTING

Seed anatomy and physiology dictate which procedures are used for TZ testing. Seeds are physiologically prepared (preconditioned) by imbibition which serves to hydrate the tissues and activate seed enzyme systems. The TZ reduction reaction will not proceed on dehydrated tissue. Physical preparation for staining involves cutting, piercing, scarification, or dissection to allow the TZ solution to penetrate tissues and come in contact with the respiring cells. After staining has taken place (post-staining), additional dissection or chemical clearing can aid in the evaluation of essential structures. The general procedures for conducting a TZ test are as follows:

14.1 Preconditioning

Preconditioning is the process of hydrating seed tissues before cutting or piercing. Imbibition activates enzyme systems, makes the tissues less fragile, softens the seed coat, and improves TZ uptake and staining. Preconditioning for TZ staining often mimics the initial stages of the germination test. Germination test requirements offer good clues for preparing seeds for TZ uptake. Deeply dormant seeds may stain better after employing dormancy breaking methods such as imbibition with gibberellic acid (400 ppm) and alternating temperatures (See section 6). With other seeds, preconditioning with hydrogen peroxide (0.5% concentration) helps bleach out pigmentation of translucent seed coats. There are some species that do not require any preconditioning (e.g. *Dactylis glomerata* - orchardgrass).

If seeds are preconditioned for an extended length of time, sprouting may occur, tissues will become flaccid, fungal activity will begin, and metabolic breakdown of the seed may proceed. Time and temperature guidelines must be established for each seed type to maintain a healthy preconditioned seed.

14.1.1 Slow moistening

Slow moistening is a desirable preconditioning method for the larger seeds (e.g. large-seeded legumes) that are especially dry, brittle and prone to fracturing. It is accomplished by placing the seeds on top of or in between moistened blotters, filter paper, paper toweling, or other media that do not contain free water. Seeds are moistened overnight (approximately 16 hrs.). Hydrating seeds slowly allows vital tissues to imbibe and expand without rupturing or fracturing. This method also prevents tissue death that can occur from the anaerobic conditions of full immersion. For species that germinate quickly, imbibition at 5°C to 10°C is recommended.

14.1.2 Soaking

Soaking is a faster method to precondition smaller seeds (e.g. Poaceae) that are less likely to rupture. This method decreases preconditioning time. Seeds are immersed in water for a prescribed length of time. Anaerobic conditions can cause tissue death, so attention to immersion time is crucial.