# List of Committee Members

<table>
<thead>
<tr>
<th>Role</th>
<th>Name</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chair</td>
<td>Stefanie Krämer</td>
<td>Germany</td>
</tr>
<tr>
<td>Vice-Chair</td>
<td>Ronald Don</td>
<td>United Kingdom</td>
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<tr>
<td></td>
<td>Izelle Allison</td>
<td>South Africa</td>
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<td></td>
<td>María Belén Aranguren</td>
<td>Argentina</td>
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<tr>
<td></td>
<td>Valerie Blouin</td>
<td>France</td>
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<td></td>
<td>Sharon Davidson</td>
<td>United States</td>
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<td></td>
<td>Gary Duffy</td>
<td>Ireland</td>
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<td></td>
<td>Teresita Farras</td>
<td>Uruguay</td>
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<td></td>
<td>Jose B. Franca-Neto</td>
<td>Brazil</td>
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<td></td>
<td>Kareen Hill</td>
<td>Australia</td>
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<td></td>
<td>Irena Jumburga</td>
<td>Latvia</td>
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<td></td>
<td>Linda Maile</td>
<td>United Kingdom</td>
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<td></td>
<td>Augusto Martinelli</td>
<td>Argentina</td>
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<td></td>
<td>Sergio Pasquini</td>
<td>Italy</td>
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<tr>
<td></td>
<td>Anny van Pijlen</td>
<td>Netherland</td>
</tr>
</tbody>
</table>
A RULES Development

Introduction of new species:

Table 6 A Part I:

*Allium, Cucumis, Lycopersicon, Lactuca* completed 2007

*Chloris gayana* completed 2008

Table 6 A Part II:

*Larix, Picea* completed 2009
<table>
<thead>
<tr>
<th>Species</th>
<th>Pretreatment: type/min. time (h)</th>
<th>Preparation before staining</th>
<th>Staining solution (%)</th>
<th>Optimum staining time (h)</th>
<th>Preparation for evaluation</th>
<th>Permitted non-viable tissue</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><em>Allium</em> spp.</td>
<td>W/18</td>
<td>Cut off a thin slice at the linear side of the seed and longitudinally ⅓ into the endosperm near the middle of the seed between radicle and cotyledons.</td>
<td>1</td>
<td>18</td>
<td>Cut longitudinally from the flat side through endosperm to expose the embryo.</td>
<td>None, including endosperm, except small superficial necrosis on the outer part of the endosperm, not in connection with the embryo cavity.</td>
<td>-</td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em></td>
<td>W/18</td>
<td>Cut between radicle and cotyledons ⅓ into the endosperm.</td>
<td>1</td>
<td>18</td>
<td>Cut the seed at the flat side into two halves, observe cut surfaces</td>
<td>None</td>
<td>Sometimes a staining of 42 hours gives a clearer and darker staining. The size of the embryo must be more than one half of the normal size</td>
</tr>
<tr>
<td><em>Lactuca</em> spp.</td>
<td>Prepare the dry seed, cut longitudinally ⅓ through the distal end of the fruit (achene) and W/18</td>
<td>Expose the embryo by gently pressing the seed coat.</td>
<td>1</td>
<td>3</td>
<td>Observe Embryo.</td>
<td>⅓ radicle, measured from the radicle tip, ⅕ of the distal end of the cotyledons, if superficial; ⅓ at distal end, if pervading.</td>
<td>-</td>
</tr>
<tr>
<td><em>Cucumis</em> spp.</td>
<td>W/18</td>
<td>Cut off transversally a small part of the seed at distal end. Cut lateral longitudinally</td>
<td>1</td>
<td>6</td>
<td>Observe embryo.</td>
<td>⅓ radicle, measured from the radicle tip, ⅕ of the distal end of cotyledons</td>
<td>-</td>
</tr>
</tbody>
</table>
Methods were taken from the ISTA Working Sheets for Tetrazolium Testing
Method for

Allium cepa

1. Species / Genus
   Allium, Liliaceae, Leek, Garlic, Onion, Lauch
   Seed tissue (lateral view)

2. Instruments
   Beakers (4 x 50 ml), razorblades, dissecting needle, dissecting needle (surgical tip), scalpel, support for preparation (rubber), filter paper, forceps, support for evaluation, binocular

3. Pretreatment
   Soak 18 hours in water at 20°C

4. Preparation before staining
   Cut longitudinally through the linear side of the seed (only a thin slice) and longitudinally ¾ into the endosperm near the middle of the seed between radicle and cotyledons ¼ into the endosperm.

5. Staining
   18 hours, 30°C, 1.0 % TZ-solution

6. Preparation for evaluation
   Cut longitudinally from the flat side through endosperm to expose the embryo

7. Evaluation (maximum area of unstained, flaccid and/or necrotic tissue permitted)
   None, including endosperm, except small superficial necrosis on the outer part of the endosperm, not in connection with the embryo cavity

8. Remarks
   None

Fig. 1: Preparation step (s)
Fig. 2: Evaluation, examples of non-viable seeds
Experimental errors between laboratories in the viability tests in onion (*Allium cepa*)

Validation study:
4 samples in 9 laboratories

<table>
<thead>
<tr>
<th>Seed lot / laboratory</th>
<th>Standard deviation (%)</th>
<th>Expected sd (%)</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>7.33</td>
<td>2.02</td>
<td>3.64</td>
</tr>
<tr>
<td>Lot 2</td>
<td>2.74</td>
<td>1.78</td>
<td>1.54</td>
</tr>
<tr>
<td>Lot 3</td>
<td>7.98</td>
<td>1.90</td>
<td>4.21</td>
</tr>
<tr>
<td>Lot 4</td>
<td>12.84</td>
<td>2.43</td>
<td>5.29</td>
</tr>
</tbody>
</table>
Method for

*Cucumis sativus*

(not yet included in the working sheets but ready for take off)
Experimental errors between laboratories in the viability tests in cucumber (Cucumis sativus)

Validation study:
3 samples in 6 laboratories

<table>
<thead>
<tr>
<th>Seed Lot / Laboratory</th>
<th>Standard Deviation (%)</th>
<th>Expected sd (%)</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>4.17</td>
<td>0.97</td>
<td>4.28</td>
</tr>
<tr>
<td>Lot 2</td>
<td>1.18</td>
<td>0.79</td>
<td>1.49</td>
</tr>
<tr>
<td>Lot 3</td>
<td>0.97</td>
<td>1.73</td>
<td>3.23</td>
</tr>
</tbody>
</table>
Method for

*Lactuca sativa*

1. **Species / Genus**

   *Lactuca*, Asteraceae, Lactuca, Lattich, Kopfsalat

2. **Instruments**

   Beakers (4 x 50 ml), little bowl, razorblades, dissecting needle, dissecting needle (lancet tip), scalpel, filter paper, forceps, support for evaluation, binocular

3. **Pretreatment**

   Prepare the dry seed, cut longitudinally ¼ through the distal end of the fruit (achene), soak 18 hours in water at 20°C

4. **Preparation before staining**

   Expose the embryo by gently pressing of the achene and seed coat

5. **Staining**

   6 hours, 30°C, 1.0 % TZ-solution

6. **Preparation for evaluation**

   None

7. **Evaluation (maximum area of unstained, flaccid and/or necrotic tissue permitted)**

   ¼ radicle, measured from the radicle tip, ½ of the distal end of the cotyledons, if superficial; ½ at distal end, if pervading

8. **Remarks**

   None

**Fig. 1**: Preparation step(s)

**Fig. 2**: Evaluation, examples of non-viable seeds
Experimental errors between laboratories in the viability tests in lettuce (*Lactuca sativa*)

Validation study:
4 samples in 8 laboratories

<table>
<thead>
<tr>
<th>Seed Lot / Laboratory</th>
<th>Standard Deviation (%)</th>
<th>Expected SD (%)</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>4.75</td>
<td>1.80</td>
<td>2.64</td>
</tr>
<tr>
<td>Lot 2</td>
<td>2.58</td>
<td>0.99</td>
<td>2.59</td>
</tr>
<tr>
<td>Lot 3</td>
<td>1.89</td>
<td>1.04</td>
<td>1.81</td>
</tr>
<tr>
<td>Lot 4</td>
<td>2.44</td>
<td>1.70</td>
<td>1.43</td>
</tr>
</tbody>
</table>
Method for

*Lycopersicon esculentum*

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Species / Genus</td>
<td><em>Lycopersicon esculentum</em>, Solanaceae, Tomato, Tomate</td>
</tr>
<tr>
<td>2. Instruments</td>
<td>Beakers (4 x 50 ml), razorblades, dissecting needle, dissecting needle (lancet tip), scalpel, filter paper, forceps, support for evaluation, binocular</td>
</tr>
<tr>
<td>3. Pretreatment</td>
<td>Soak 16 hours in water at 20°C</td>
</tr>
<tr>
<td>4. Preparation before staining</td>
<td>Cut between radicle and cotyledons ½ into the endosperm</td>
</tr>
<tr>
<td>5. Staining</td>
<td>16 hours, 30°C, 1.0% TZ-solution</td>
</tr>
<tr>
<td>6. Preparation for evaluation</td>
<td>Cut the seed at the flat side into two halves, observe cut surfaces</td>
</tr>
<tr>
<td>7. Evaluation (maximum area of unstained, flacid and/or necrotic tissue permitted)</td>
<td>None</td>
</tr>
<tr>
<td>8. Remarks</td>
<td>Sometimes a staining of 42 hours gives a clearer and darker staining. The size of the embryo must be more than one half of the normal size</td>
</tr>
</tbody>
</table>

Fig. 1: Preparation step(s)

Fig. 2: Evaluation, examples of non-viable seeds
Experimental errors between laboratories in the viability tests in tomato (*Lycopersicon esculentum*)

Validation study: 4 samples in 9 laboratories

<table>
<thead>
<tr>
<th>Seed Lot / Laboratory</th>
<th>Standard Deviation (%)</th>
<th>Expected SD (%)</th>
<th>F-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>6.52</td>
<td>1.75</td>
<td>3.70</td>
</tr>
<tr>
<td>Lot 2</td>
<td>1.90</td>
<td>0.88</td>
<td>2.17</td>
</tr>
<tr>
<td>Lot 3</td>
<td>7.11</td>
<td>2.02</td>
<td>3.51</td>
</tr>
<tr>
<td>Lot 4</td>
<td>2.15</td>
<td>1.75</td>
<td>1.23</td>
</tr>
</tbody>
</table>
Conclusions:

Variation between replicates within the tests was within tolerance (table 5.1, data not shown).

Variation between laboratories was mainly good. Even in *Allium* with high f-values, the results were within tolerated ranges calculated according to Miles.

Thus, reproducibility was proven to be sufficient.

The four methods are proposed as ISTA Rules Changes 2008.
Thanks to the participants of the comparative tests

- **Maria Belén Aranguren**, Dow Agro Sciences, Argentina
- **Augusto Martinelli**, Rayen Laboratories, Argentina
- **Valerie Blouin**, GEVES-SNES, France
- **Stefanie Krämer**, LTZ Augustenborg, Germany
- **Benita Derilo** and **Irene Jumburga** National Seed Testing Laboratory, Latvia
- **Anny van Pijlen**, NAK, The Netherlands
- **Linda Maile**, CPVS NIAB, UK
- **Ronald Don** and **Caroline Cadger**, SASA, Scotland
- **Annette Miller**, USDA/ARS, USA

and to various seed companies for providing seed samples as well as to the TEZ Committee Chair Stefanie Krämer
B Publications

Reorganisation of Tetrazolium Handbook
  Update of information
  Inclusion of theoretical background
  Rearrangement of test methods  completed 2010

Continue Tetrazolium Working Sheets Part I  completed 2007
  52 new species

Continue Tetrazolium Working Sheets Part II  completed 2007
  16 new species

Revision of ISTA Tetrazolium Working Sheets  completed 2008
Continue Tetrazolium Working Sheets Part I

Abutilon
Adonis
Ageratum houstonianum
Allium ursinum
Angelica
Antirrhinum
Aster
Barbarea
Bellis perennis
Callistephus chinensis
Catharanthus
Cicer arietinum
Cichorium
Cirsium arvense
Coreopsis grandiflora
Coreopsis tinctoria
Corynephorus canescens
Cosmos
Cuscuta
Dahlia
Euphorbia
Fallopia
Foeniculum
Gazania
Gentiana lutea
Gentiana septemfida
Glechoma
Helichrysum
Lathyrus
Lens culinaris
Leontodon
Lepidium
Lepidium sativum
Meconopsis
Origanum
Passiflora
Perilla
Persicaria lapathifolia
Pimpinella
Polygonum
Rhinanthus
Ricinus communis
Rudbeckia hirta
Salvia
Securigera varia
Spinacia oleracea
Urtica
Valeriana officinalis
Valerianella
Zinnia
Continue Tetrazolium Working Sheets Part II

Betula
Chamaedorea elegans
Chamaedorea humilis
Chimonanthus praecox
Livistona
Musa
Pachypodium
Panax ginseng
Phoenix
Pittosporum
Ruscus
Sequoiadendron giganteum
Strelitzia
Trachycarpus fortunei
Yucca
Zelkova
C Workshops and Seminars

June 2008   Italy            In cooperation with FTS
             ISTA FTS Workshop in Peri

July 2008   Argentina         Purity, Germination and Tetrazolium Test on
                                Tropical and Subtropical Seeds

2009       Brazil            Tropical and sub tropical Tree Seeds

2010       Germany           Germination and Tetrazolium Workshop
                                (before the ISTA Congress)
<table>
<thead>
<tr>
<th>Date</th>
<th>Country</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 2008</td>
<td>Brazil</td>
<td>Question about Toleranz tables in TEZ testing</td>
</tr>
<tr>
<td>March 2008</td>
<td>Austria</td>
<td>Question about Preparation of Colchicum autumnale spp.</td>
</tr>
<tr>
<td>May 2008</td>
<td>Spain</td>
<td>Questions about the Vakuum equipment</td>
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</table>
D Proficiency Tests

2007  *Medicago sativa*

2008  *Lolium*

2009 and 2010  see PTC
Thank you