SECTION A - GENERAL INFORMATION

1 PURPOSE

1.1 This Protocol sets out the procedures for conducting tests and assessments in relation to official examinations of DUS, maintenance of reference stocks and verification of VCU submissions of varieties of winter and spring Barley entered for National List (NL) Trials and Plant Breeders’ Rights (PBR).

2 SCOPE

2.1 These procedures apply to all varieties of Barley. Special procedures and responsibilities for Genetically Modified (GM) varieties are set out in Sections A5 and A6.

2.2 Except where specified in this protocol or authorised by APHA Varieties and Seeds, only National List candidates, Plant Breeders’ Rights candidates, candidates for Foreign Authorities and the reference varieties may be incorporated in the DUS tests.

3 RESPONSIBILITIES

3.1 The growing tests and assessments in this protocol are carried out under the responsibility of the Secretary of State for Environment, Food and Rural Affairs, Scottish Ministers, the Welsh Ministers and the Minister for Agriculture and Rural Development in Northern Ireland (the National Authorities).

3.2 They are supervised, on behalf of the National Authorities, by officials of the Testing Authorities, that is, the Animal and Plant Health Agency (APHA), Scottish Government Agriculture and Rural Development Division (SGARD), the Department of Agriculture and Rural Development for Northern Ireland (DARDNI) and the Welsh Government (WG).

3.3 This protocol is authorised by the Plant Variety and Seeds Committee (PVSC). It cannot be amended without their approval. Requests and suggestions for amendment of the protocol should be put in writing to APHA Varieties and Seeds, either directly or via the Test Centre.

3.4 The procedures are administered by:

1.1.1. Plant Varieties and Seeds
The Animal and Plant Health Agency
Eastbrook
Shaftesbury Road
Cambridge Tel No 0300 060 0497
CB2 8DR Fax No 0300 060 2115

3.5 TEST CENTRE

The DUS growing tests and assessments in this protocol are co-ordinated and carried out by the:

Centre for Plant Varieties and Seeds (Test Centre)
NIAB
Huntingdon Road
Cambridge Tel No. 01223 342200
CB3 0LE Fax No. 01223 277602

3.6 The Test Centre is responsible for providing the appropriate facilities.
4 NON-COMPLIANCE WITH THE PROTOCOL

4.1 Where the protocol uses the word “must” for any action then failure to carry out this action will result in non-compliance. Where non-compliance occurs or there are concerns regarding the validity of any data or tests this must be reported to APHA. Where this protocol uses the word “should” for any action this is the method to be followed unless there are clear reasons which can be justified by the Test Centre as technically sound.

5 RESPONSIBILITY FOR GM RELEASES

5.1 GM Release Consent Holders are responsible for GM releases. All parties involved in DUS work operating under a GM Release Consent must adhere to the instructions of the Release Consent Holder, where necessary, to comply with the relevant consent conditions. Where DUS protocol non-compliance occurs, this must be reported to the consent holder and the Test Centre who will notify APHA.

6 PROCEDURES FOR GM VARIETIES

6.1 Applicants intending to enter GM candidates must consult APHA, well in advance of their application, about specific requirements under GM regulations.

6.2 The Test Centre must ensure that no test or trial sites are planted with GM candidates and/or varieties until APHA has given the specific clearances.

7 ASSOCIATED DOCUMENTS

7.1 The following documents are associated with this protocol

<table>
<thead>
<tr>
<th>Reference</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley VCU Protocol</td>
<td>United Kingdom National List Trials: Protocol and Procedures for Examining the Value for Cultivation and Use (VCU) of Cereals (wheat, barley, oats, triticale, rye and spelt wheat)</td>
</tr>
<tr>
<td>UPOV TG/1/3</td>
<td>General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonised Descriptions of New Varieties of Plants. 19.04.2002</td>
</tr>
<tr>
<td>UPOV TGP/8/1</td>
<td>Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability. 21.10.2010</td>
</tr>
<tr>
<td>UPOV TGP/9/1</td>
<td>Examining Distinctness. 11.4.2008</td>
</tr>
<tr>
<td>UPOV TGP/10/1</td>
<td>Examining Uniformity. 30.10.2008</td>
</tr>
<tr>
<td>UPOV TG19/10</td>
<td>Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Barley. 04.11.1994</td>
</tr>
</tbody>
</table>
SECTION B - APPLICATION REQUIREMENTS

1 PURPOSE

1.1 The purpose of this section is to identify the specific requirements for National List and Plant Breeders’ Rights applications.

2 SCOPE

2.1 These procedures apply to all applications.

3 RESPONSIBILITIES

3.1 The applicants are responsible for ensuring that these procedures are complied with.

4 RECEIPT OF APPLICATIONS

4.1 The latest date for receipt of applications for acceptance of a variety onto the National List or for Plant Breeders’ Rights, which is set administratively by APHA, is 31st August for Winter Barley and 30th November for Spring Barley. Applications received after this date may be considered for inclusion in the current year’s tests and trials on a case-by-case basis.

4.2 The procedures for the submission of National List and Plant Breeders’ Rights applications, technical questionnaires and for payment of administration fees can be obtained from APHA PVS at the address shown in Section A.

4.3 Applicants should notify APHA of special DUS characteristics which may require additional examinations. These claims should, in addition, be noted in the TQ accompanying the application.

4.4 In the case of hybrid varieties the TQ must include details of the nature of the hybrid and all progenitor lines.

5 RECEIPT OF SEED

5.1 The latest date for receipt of seed is 15th September for Winter Barley and 15th January for Spring Barley and is set administratively by APHA. Seed submissions received after this date will normally be refused. Instructions for the delivery of seed will be made available to applicants by APHA.

6 SEED QUALITY REQUIREMENTS

6.1 The seed must satisfy the quality requirements for Basic Seed as laid down in Annex II of Council Directive 66/402/EC, as amended, on the marketing of cereal seed..

6.2 The seed must not be chemically treated. Seed treatment, if required, will be undertaken by the Test Centre. The chemicals applied and rates of application will be determined by the Test Centre.
## SEED QUANTITY

1. Conventional Type
   - 1.5 kg bulk seed*
   - 0.5 kg selected seed with 1000 seed weight given

2. Single Cross Hybrids
   **Hybrid**
   - 1.5 kg bulk seed*
   - 0.5 kg selected seed with 1000 seed weight given
   **Male sterile (female parent)**
   - 1.5 kg bulk seed*
   - 0.5 kg selected seed with 1000 seed weight given
   **Pollinator (male parent)**
   - 1.5 kg bulk seed*
   - 0.5 kg selected seed with 1000 seed weight given
   - Parents already on the UK National List or with UK PBR seed need not be supplied

3. Other Types
   - Contact APHA

*There is a separate submission of seed for VCU trials in Year 1.

### 7.1 Year 2 and Further Year Submissions

None for DUS

A sample of 350gms of seed will be drawn from the VCU submissions in Year 2 and any further years to authenticate the submission (see Section I). Applicants should refer to associated document APHA VCU Cereals for Year 2 VCU seed requirements and Section H4.2 dealing with replacement seed of a variety.

### 7.2 Shortfall in Seed Quantities

Where insufficient seed is available in the first instance a further stock should be supplied in the following year which will be authenticated against the original submission. An additional charge may be applied.

### 7.3 Hybrid Barley

In the case of hybrid Barley where insufficient seed stocks of parent lines are available, a minimum of 200g of each line should be supplied in the first instance. Further stocks should be supplied in the following year and these will be authenticated against the original submission for which an additional charge may be applied. Where components of hybrids are on the UK National List, or have UK PBR, seed need not be supplied unless specifically requested.
8 LABELLING REQUIREMENTS, INCLUDING PROVISIONS FOR GM VARIETIES

8.1 Applicants **must** clearly label their seed, inside and outside the bag, with the following information:

- Applicant
- Breeder’s Reference number or name
- Type of Seed (Combined submission of DUS and VCU)
- Quantity of seed
- Whether it is a parental line

8.2 All packages of GM material must be clearly labelled as “GMO” or "Genetically Modified Organism".
SECTION C – GROWING TEST PROCEDURES

1 PURPOSE

1.1 The purpose of this section is to provide details of the procedures used in the growing tests for DUS analysis.

2 SCOPE

2.1 These procedures apply to all varieties of Barley.

3 RESPONSIBILITIES

3.1 The Test Centre is responsible for conducting these procedures.

3.2 The Test Centre will be responsible for ensuring that no material supplied to them is used for any other purpose than the conduct of these procedures or the release of reference samples for authorised purposes. (See Section E 6).

4 REFERENCE VARIETIES

4.1 The principles governing the selection of reference varieties are set out in Appendix 1.

4.2 Seed of reference varieties will be supplied by the Test Centre.

5 DESIGN OF TESTS

5.1 The Test Centre is responsible for selecting a suitable site which should be on ground that has not normally had a barley crop in the previous three years but may be less where the risk of contamination is negligible.

5.2 Field husbandry should follow best local practice for all operations and particularly as regards cultivation, drilling, fertiliser and spray application, use of irrigation and control of pests and diseases.

5.3 The minimum duration of tests should normally be two independent growing cycles. Additional growing cycles may be approved by the NLSC.

5.4 From information given in the Technical Questionnaire the candidate variety may be grown in field grown plots and compared with varieties which are in the same classification for the following characters; number of rows in the ear; rachilla hair type of the grain; presence or absence of hairs in the ventral furrow of the grain; for male sterile parent lines as components of hybrid varieties: presence or absence of pollen production; seasonal type.

Pollen production – absence or presence will be used as a UK grouping character. It is tested by growing the male sterile parent line in isolated plots of approx. 2000 plants.

5.5 Plots are sown from the submitted seed in the first year of test as follows (each year of test).

<table>
<thead>
<tr>
<th>Number of plots:</th>
<th>2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number of Plants Examined/Variety:</td>
<td>2000 (approx)</td>
</tr>
<tr>
<td>No. of plants /m²</td>
<td>200-300</td>
</tr>
<tr>
<td>Time of sowing (winter Barley)</td>
<td>October - November</td>
</tr>
<tr>
<td>Time of sowing (spring Barley)</td>
<td>February – April</td>
</tr>
</tbody>
</table>
5.6.1 * One plot of 9 x 1.6 m with six rows are sown. Three of the six rows are sown with the selected seed and authenticated against the other three rows sown with seed from the 1.5kg bulk submission. The first replicate is organised in AFP number order. CPVO grouping characters are used to sow the second replicate of 9mx1.6m with six rows: three rows per candidate and two candidates per plot.

5.6.2 In the case of winter varieties an additional plot is sown in late April from the selected seed during the first year of tests, to examine the uniformity of the vernalisation response of the variety under test. The assessment for the characteristic “seasonal type” should be carried out on at least 500 plants

Number of plots: 1 *
Total Number of Plants Examined/Variety: minimum 500 plants (approx)
No. of plants /m² 200-300
Time of sowing (winter wheat) Late April

*One plot 9m x 1.6 m with six rows is sown, with three rows per candidate and two candidates per plot

In the case of winter hybrid varieties, the uniformity of the vernalisation response is tested on a single seed plot of minimum. 500 plants in the first year of tests.

5.7 During the second year of testing, plots are sown from the 'selected' seed and a second replicate of three rows using seed from the bulk submission

Number of plots 1 ½
Total number of plants examined/ Variety 2000 (approx)
No. of plants/m² 200-300
Time of sowing (winter barley) October – November
Time of sowing (spring barley) February – April

5.8 At the end of the first recording year, varieties in test with possible distinctness problems are identified and in the second year of test may be sown in direct comparison plots. At the end of the second year of test candidate varieties that are still not distinct may be grown in additional direct comparison plots for which an additional charge will be made to the applicant.

6 RECORDS AND RECORDING

6.1 All records and plot data should be in a form determined and validated by the Test Centre.

6.2 Characters, recording details and instructions are given in Section D. Any variant and abnormal plants or plants resulting from an adverse reaction to husbandry practice are recorded but excluded from the sample.

6.3 In the first recording year, characters, as indicated in Section C5.1, are recorded on all varieties in test and the data analysed to assess uniformity of the candidate variety and to determine the most similar reference varieties. (For details see Section G.)

6.4 In the second recording year, characters, as indicated in Section C5.1, are assessed on all varieties in test and the data analysed and, together with those from the first year, used to assess distinctness and uniformity of the candidate variety. (For details see Section G.)

6.5 If the Test Centre notices unusual or novel characters in candidate varieties they may be noted and a photographic record taken.
7 COMMUNICATIONS WITH THE APPLICANT

7.1 The Test Centre will notify the applicant or the agent of any DUS problems at the earliest practical opportunity as they arise during the growing season. All such notifications are copied to APHA. If DUS problems arise applicants will be invited to visit the DUS tests by arrangement so that the material can be examined and discussions held with the experts at the Test Centre.

APHA will communicate with the Test Centre any action as a result of that notification.

7.2 If confidentiality considerations allow, the applicant should be informed which variety is similar and be invited to submit any information which may help to distinguish them.

7.3 After each recording season the results are summarised and reported to the applicant and APHA by the Test Centre.
SECTION D - SUMMARY OF DUS CHARACTERISTICS TO BE ASSESSED, METHOD OF ASSESSMENT AND STANDARDS APPLIED

1 PURPOSE

1.1 The purpose of this section is to summarise the characteristics to be assessed.

2 SCOPE

2.1 This section summarises characteristics, states of expression, method of observation and standards required for DUS assessment.

3 RESPONSIBILITIES

3.1 The Test Centre is responsible for co-ordinating the procedures in this summary.

4 ORGANISATION

4.1 The minimum duration of tests to assess characteristics is normally two consecutive growing periods. Shorter periods may be applied for assessment of additional characteristics. Additional growing periods must be approved by the UK National List and Seeds Committee.

5 DUS CHARACTERISTICS TO BE ASSESSED

5.1 Routine Characteristics

The following table summarises the DUS characteristics to be routinely examined.

Note: * denotes a characteristic which must be examined according to European Commission Directive 2003/90/EC, the CPVO protocol and/or UPOV Guidelines.

G denotes a grouping characteristic

D denotes a characteristic used in the variety description
<table>
<thead>
<tr>
<th>CPVO-TP 019/3</th>
<th>UPOV TG/19/10</th>
<th>Character</th>
<th>Material examined</th>
<th>Number of plants or sample size for assessment</th>
<th>Method of assessment and recording</th>
<th>States of expression</th>
<th>D Method Minimum Difference required UPOV TG/19/10</th>
<th>U Method UPOV TC/34/5</th>
</tr>
</thead>
</table>
| 1            | 28            | Kernel: colour of aleurone layer | Submitted seed and ears | 20 grain sample | Visual score | 1 whitish  
2 weakly coloured  
3 strongly coloured | 2 states | 1% @95% |
| 2            | 1             | Plant: Growth habit | DUS plot | 2000 plants | Visual score | 1 erect  
3 semi-erect  
5 intermediate  
7 semi-prostrate  
9 prostrate | 2 - 3 states | 0.1% @95% |
| 3            | 2             | Lowest Leaves: hairiness of leaf sheaths | DUS plot | 20 plants sample | Visual score | 1 absent  
9 - present | 1 state | 1% @95% |
| 4            |               | Flag leaf: intensity of anthocyanin colouration of auricles | DUS plot | 2000 plants | Visual score | 1 absent or very weak  
3 weak  
5 medium  
7 strong  
9 very strong | 2 - 3 states | 0.1% @95% |
| 5            | 5             | Flag leaf: attitude | DUS plot | 2000 plants | Visual score | 1 erect  
3 semi-erect  
5 horizontal  
7 semi-drooping  
9 drooping | 2 - 3 states | 0.1% @95% |
| 6            | 6             | Flag leaf: glaucosity of sheath | DUS plot | 2000 plants | Visual score | 1 absent or very weak  
3 weak  
5 medium  
7 strong  
9 very strong | 2 - 3 states | 0.1% @95% |
| 7            | 7             | Time of ear emergence: (first spikelet visible on 50% of ears) | DUS plot | 2000 plants | Data converted to a score | 1 very early  
3 early  
5 medium  
7 late  
9 very late | 2 - 3 states | 0.1% @95% |
| 8            |               | Awns: intensity of anthocyanin colouration of tips | DUS plot | 2000 plants | Visual score | 1 absent or very weak  
3 weak  
5 medium  
7 strong  
9 very strong | 2 - 3 states | 0.1% @95% |
| 9            | 10            | Ear: glaucosity | DUS plot | 2000 plants | Visual score | 1 absent or very weak  
3 weak  
5 medium  
7 strong  
9 very strong | 2 - 3 states | 0.1% @95% |
| 10           | 11            | Ear: attitude | DUS plot | 2000 plants | Visual score | 1 erect  
3 semi-erect  
5 horizontal  
7 semi-recurved  
9 recurved | 2 - 3 states | 0.1% @95% |
<table>
<thead>
<tr>
<th>CPVO-TP 019/3</th>
<th>UPOV TG/19/10</th>
<th>Character</th>
<th>Material examined</th>
<th>Number of plants or sample size for assessment</th>
<th>Method of assessment and recording</th>
<th>States of expression</th>
<th>D Method Minimum Difference required UPOV TG/19/10</th>
<th>U Method UPOV TC/34/5</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>12</td>
<td>Plant: length (stem, ear and awns)</td>
<td>DUS plot</td>
<td>2000 plants</td>
<td>Measured and given score</td>
<td>1 very short 3 short 5 medium 7 long 9 very long</td>
<td>2 - 3 states</td>
<td>0.1% @95%</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>Ear: number of rows</td>
<td>DUS plot</td>
<td>2000 plants</td>
<td>Visual score</td>
<td>1 two-row 2 more than two</td>
<td>1 state</td>
<td>0.1% @95%</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>Ear: shape</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>3 tapering 5 parallel 7 fusiform</td>
<td>2 states</td>
<td>Sample</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
<td>Ear: density</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 very lax 3 lax 5 medium 7 dense 9 very dense</td>
<td>2 - 3 states</td>
<td>1% @95%</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>Ear: length (excluding awns)</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 very short 3 short 5 medium 7 long 9 very long</td>
<td>2 - 3 states</td>
<td>1% @95%</td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td>Awn: length (compared to ear)</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>3 short 5 medium 7 long</td>
<td>2 states</td>
<td>1% @95%</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>Rachis: length of first segment</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>3 short 5 medium 7 long</td>
<td>2 - 3 states</td>
<td>1% @95%</td>
</tr>
<tr>
<td>18</td>
<td>19</td>
<td>Rachis: curvature of first segment</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 absent or very weak 3 weak 5 medium 7 strong 9 very strong</td>
<td>2 - 3 states</td>
<td>1% @95%</td>
</tr>
<tr>
<td>19</td>
<td>20&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Ear: development of sterile spikelets</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 none or rudimentary (&quot;deficiens&quot;) 2 full</td>
<td>1 state</td>
<td>1% @95%</td>
</tr>
<tr>
<td>20</td>
<td>20&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Sterile spikelet: attitude (in mid-third of ear)</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 - parallel 2 - parallel to weakly divergent 3 - divergent</td>
<td>2 states</td>
<td>1% @95%</td>
</tr>
<tr>
<td>21</td>
<td>21</td>
<td>Median spikelet: length of glume and its awn relative to grain</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 shorter 2 equal 3 longer</td>
<td>1 state</td>
<td>1% @95%</td>
</tr>
<tr>
<td>CPVO-TP 019/3</td>
<td>UPOV TG/19/10</td>
<td>Character</td>
<td>Material examined</td>
<td>Number of plants or sample size for assessment</td>
<td>Method of assessment and recording</td>
<td>States of expression</td>
<td>D Method Minimum Difference required UPOV TG/19/10</td>
<td>U Method UPOV TC/34/5</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------</td>
<td>-----------</td>
<td>------------------</td>
<td>---------------------------------------------</td>
<td>----------------------------------</td>
<td>---------------------</td>
<td>---------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>22</td>
<td>22</td>
<td>Grain: rachilla hair type</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 short or 2 long</td>
<td>1 state</td>
<td>1% @95%</td>
</tr>
<tr>
<td>23</td>
<td>23</td>
<td>Grain: husk</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 - absent or 2 - present</td>
<td>1 state</td>
<td>1% @95%</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
<td>Grain: anthocyanin colouration of nerves of lemma</td>
<td>DUS plot</td>
<td>2000 plants</td>
<td>Visual score</td>
<td>1 absent or very weak or 2 - weak or 3 - weak or 5 - medium or 7 - strong or 9 - very strong</td>
<td>2 - 3 states</td>
<td>0.1% @95%</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>Grain: spiculation of inner lateral nerves of dorsal side of lemma</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 absent or very weak or 2 - weak or 3 - weak or 5 - medium or 7 - strong or 9 - very strong</td>
<td>2 - 3 states</td>
<td>1% @95%</td>
</tr>
<tr>
<td>26</td>
<td>26</td>
<td>Grain: hairiness of ventral furrow</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 absent or 2 - present</td>
<td>1 state</td>
<td>1% @95%</td>
</tr>
<tr>
<td>27</td>
<td>27</td>
<td>Grain: disposition of lodicules</td>
<td>DUS plot</td>
<td>20 plant sample</td>
<td>Visual score</td>
<td>1 frontal (“bib” type) or 2 clasping (“collar” type)</td>
<td>1 state</td>
<td>1% @95%</td>
</tr>
<tr>
<td>28</td>
<td>29</td>
<td>Seasonal type</td>
<td>DUS plot</td>
<td>Winter and alternative types: 1000 plant plot test sown in late spring or Spring types: TQ declaration</td>
<td>Visual score</td>
<td>1 winter type or 2 alternative type or 3 spring type</td>
<td>2 states</td>
<td>1% @95%</td>
</tr>
</tbody>
</table>
### Special Category Characteristics

These characters should only be used as a complement to confirm other differences morphological or physiological differences.

<table>
<thead>
<tr>
<th>CPVO-TP 019/2 rev</th>
<th>UPOV TG/19/10</th>
<th>UK</th>
<th>Character</th>
<th>Material examined</th>
<th>Number of plants or sample size for assessment</th>
<th>Method of assessment and recording</th>
<th>States of expression</th>
<th>D Method Minimum Difference required UPOV TG/19/10</th>
<th>U Method UPOV TC/34/5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>93</td>
<td>D-Hordein composition: allele expression at locus Hor-3</td>
<td>Submitted seed</td>
<td>20 grains for Distinctness 100 grains for Uniformity</td>
<td>Visual score</td>
<td>1 state</td>
<td>1% @95% see note below</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31</td>
<td>94</td>
<td>C-Hordein composition: allele expression at locus Hor-1</td>
<td>Submitted seed</td>
<td>20 grains for Distinctness 100 grains for Uniformity</td>
<td>Visual score</td>
<td>1 state</td>
<td>1% @95% see note below</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>95</td>
<td>B-Hordein composition: allele expression at locus Hor-2</td>
<td>Submitted seed</td>
<td>20 grains for Distinctness 100 grains for Uniformity</td>
<td>Visual score</td>
<td>1 state</td>
<td>1% @95% see note below</td>
</tr>
</tbody>
</table>

Note – allowance is made for the presence of biotypes
5.3 CPVO Approved Additional Characteristics

The following table summarises the additional characteristics which have been approved by the CPVO for European Plant Breeders’ Rights:

<table>
<thead>
<tr>
<th>Type of expression (QL, PQ, QN)</th>
<th>Characteristic</th>
<th>Growth Stage</th>
<th>Method of observation (VG, VS, MG, MS)</th>
<th>States of expression (at least two)</th>
<th>Example varieties</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>QL</td>
<td>Production of pollen (male sterility)</td>
<td>60 - 65</td>
<td>VS</td>
<td>absent, present</td>
<td>FM 99-18, MT 99-18</td>
<td>1</td>
</tr>
</tbody>
</table>

5.4 UK Additional DUS Characteristics

There are other additional characteristics which have been used by the UK in the past, but which are not accepted by the CPVO. It might be possible to use these characters in decisions for NL and UK PBR but without detailed discussion and eventual acceptance by the CPVO any DUS reports using these characters will not be accepted for EU Plant Breeders Rights. For further information please contact APHA Tel. No.01223 342396. A fee may be charged for examination of these characteristics as advised by APHA.

Applicants can suggest new additional characters on the Technical Questionnaire for testing DUS or after notification by the DUS Test Centre of distinctness problems. (For procedures see Section F).
SECTION E - REFERENCE SEED STOCK MAINTENANCE AND VCU SEED STOCK AUTHENTICATION PROCEDURES

1 PURPOSE

1.1 This section sets out the procedures for reference seed stock maintenance and VCU seed stock authentication.

2 SCOPE

2.1 These procedures apply to all reference collection varieties and VCU seed submissions where the VCU seed has not been taken from the same bulk as the seed used for the DUS test.

3 RESPONSIBILITIES

3.1 The Test Centre is responsible for conducting these procedures.

4 PROCEDURES FOR REFERENCE SEED STOCK MAINTENANCE

4.1 Subject to meeting the required quality standards a small portion of the seed is sown for observations and measurements. The seed sample submitted with the successful or pending application is considered to be the definitive seed of the variety. The remainder is stored under controlled and monitored refrigerated conditions as part of the official reference collection.

4.2 If during the normal tests there is any evidence that seed is deteriorating in storage, or that stocks are under 200 grams, a request will be made to the maintainer asking for a replacement seed (1 Kg) of the variety. This replacement seed must be authenticated against the definitive seed. Plots will be established from any replacement reference seed sample to be authenticated and compared with the definitive seed over a maximum of two recording cycles.

4.3 Plots will be established from any replacement reference seed sample to be authenticated and compared visually with the definitive seed over a maximum of two recording seasons. Plots must be examined through all the growth stages from early habit to full harvest ripeness. If the new seed sample cannot be visually distinguished from the reference seed it will be accepted as representing the variety. It will then be considered as the definitive seed and substituted for the existing definitive seed in the reference collection.

4.4 In the event of the replacement sample not meeting the required acceptance standards, an additional replacement sample will be requested. Plots will be established from any additional replacement seed sample and compared over a maximum of two recording seasons. If the additional replacement sample does not meet the acceptance criteria set out in 4.3, the variety will be deleted from the reference collection.
5 PROCEDURES FOR VCU SEED STOCK AUTHENTICATION

5.1 A representative sub-sample of seven grains from the VCU seed submission are compared to a representative sub-sample of seven grains from the DUS seed submission (definitive seed) by electrophoresis using the SDS PAGE method. The electrophoresis tests are conducted between the receipt of the submission and the date of sowing. If the VCU seed sample tested by the electrophoresis method does match the DUS seed, it will be considered to represent the variety.

5.2 If the VCU seed sample does not match the DUS seed sample a further test of 56 grains will be carried out. If the VCU seed sample still does not match the DUS seed a single plot of the VCU seed submission will be sown immediately adjacent to the DUS seed submission to verify this result in a comparison of side-by-side field sown plots. If the VCU plot does not differ from the DUS plot in the comparison of field sown plots the VCU seed will be considered to represent the variety.

5.3 Electrophoresis test is as follows:

<table>
<thead>
<tr>
<th>No of seeds tested</th>
<th>up to 56 (plus the original 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Sodium dodecyl polyacrylamide gel electrophoresis (SDS PAGE)</td>
</tr>
<tr>
<td>Time of testing</td>
<td>mid September – mid October (winter crop)</td>
</tr>
<tr>
<td></td>
<td>mid January – end February (spring crop)</td>
</tr>
</tbody>
</table>

6 PROCEDURES FOR THE INCLUSION OF NEW COMMON KNOWLEDGE VARIETIES INTO THE REFERENCE COLLECTION

6.1 When a new variety enters into common knowledge such that it must be included in the reference collection, a request will be sent by the Test Centre to the Testing Authority which has added this variety to its National list for the supply of at least 200g of seed of the definitive sample. This seed will then be used to validate a larger sample of seed from the breeder or the VCU seed sample.

7 RELEASE OF REFERENCE SAMPLES FOR AUTHORISED PURPOSES

7.1 A maximum of 200g of seed of reference samples can be supplied by the Test Centre, on request, to UK, EU and UPOV, DUS Testing Authorities and UK, EU and OECD Seed Certification Agencies. The recipient will be notified in writing that this material, or any material derived from it, must not be supplied to a Third party or used for any other purpose than as a reference for official DUS testing or seed certification.

7.2 Provision of reference samples, other than in 7.1, to any other parties must be authorised by APHA.
SECTION F - PROCEDURES FOR ASSESSMENT OF NEW ADDITIONAL DUS CHARACTERS

1 PURPOSE

1.1 This Section sets out the procedures for assessment of new additional DUS characters for varieties of Barley entered for National List trials and PBR.

2 SCOPE

2.1 These procedures apply to applications where new additional DUS characteristics which have not been approved by the NLSC are requested for use for determinations of DUS.

3 RESPONSIBILITIES

3.1 The Test Centre is responsible for communicating with the applicant to produce a proposed procedure for the conduct of new tests. This procedure must ensure that Distinctness, Uniformity and Stability will be assessed.

3.2 All new additional characteristics must be authorised by the National List and Seeds Committee.

4 REFERENCE VARIETIES

4.1 The reference varieties will include only those varieties from which the candidate variety is not distinct, as well as other varieties for control purposes.

4.2 Seed of reference varieties will be supplied by the Test Centre.

5 PROCEDURES

5.1 Details of the proposed special test or assessments will be submitted to the NLSC to consider the feasibility of setting up a test acceptable to the UK Authorities. The applicant will be advised by APHA of arrangements and costs.

5.2 The NLSC will consider the results of the commissioned test or trial when reaching its recommendation on the granting of Plant Breeders’ Rights and/or National Listing.

5.3 Where the test for a character is approved by the NLSC it should be subsequently listed in Section D5.1, 5.2 as appropriate.
SECTION G - PROCEDURES FOR DUS DECISIONS

1 PURPOSE

1.1 This section sets out the procedures for assessing DUS decisions on varieties of Barley.

2 SCOPE

2.1 These procedures apply to all varieties of Barley entered for National List and Plant Breeders’ Rights tests and those being tested for Foreign Authorities.

3 RESPONSIBILITIES

3.1 The Test Centre is responsible for applying the criteria for DUS, set out in this procedure.

3.2 The Test Centre is responsible for producing the DUS report in accordance with these procedures and for ensuring that they are in accordance with CPVO protocols or UPOV guidelines as appropriate.

4 REFERENCE VARIETIES

4.1 Appendix 1 sets out which varieties are considered as reference varieties for these procedures.

4.2 Descriptions of reference collection varieties are maintained on a database. Descriptions of the varieties in test are compared to this database of descriptions.

5 DISTINCTNESS

5.1 In accordance with associated document UPOV TG/1/3, varieties can be considered distinct where they have a different expression in a grouping character e.g. seasonal type; presence or absence of hairs on the lower leaf sheaths; presence or absence of pigment in the tips of the awns; number of rows in the ear; rachilla hair type of the grain; presence or absence of hairs in the ventral furrow of the grain; for male sterile parent lines as components of hybrid varieties: presence or absence of pollen production; seasonal type.

5.2 The standard applied for distinctness over two years of test is a significant difference of one, two or three states in the expression of a characteristic in accordance with the table of characteristics given above in Section D.

5.3 Where a statistical test is used then a standard of significant differences, using the one year “t” criterion, at 5%, in both years of test (the “2 @ 5% standard”) or, two out of three years of test (with the significant difference in the same direction).

5.4 The standard applied over additional years of tests is as 5.2. Where a statistical test is used, then a standard of significant differences of two out of three years of test with the significant difference in the same direction is used.

5.5 Where varieties are grown in close proximity under the same conditions, and a direct comparison can be made, distinctness can be determined on the basis of visual observation. Characters are recorded using the notes to represent states of expression (See Section D). In these circumstances the basis for distinctness will be clearly recorded.
5.6 Hybrids

Distinctness follows the principle of “hybrid first” i.e., if the final hybrid variety is not distinct then distinctness is examined by testing the parent lines—either the CMS (female parent) or the Restorer (pollen donor/male parent) must be clearly distinguishable from the respective male or female parent of the non-Distinct hybrid variety. Hybridity will be used as a grouping character based on the TQ declaration made by the applicant. CMS female parent lines will only be compared to other CMS lines. Pollen production—absence or presence—will also be used as a UK grouping character. It is tested by growing the male sterile parent line in isolated seed plots of approx. 2000 plants.

6 UNIFORMITY

6.1 Uniformity is assessed for all characteristics used to establish Distinctness.

6.2 Any variant plants identified by visual assessment are recorded and counted and their proportions calculated. Variants are defined as plants which are clearly not of the variety. Care is taken to ensure that the plants which are counted are not the result of any non-genetic factors such as environment, pest or disease or husbandry.

6.3 Uniformity is assessed principally in Year 1 of tests by an examination of the selected seed and the examination of the plots grown from the submitted seed. The selected seed may have all plants showing a character(s) not of the variety or may be mixed in the expression of characters that are not of the variety. The late spring sown vernalisation plots are also checked for uniformity (for a tendency to ear).

6.4 Uniformity Standards

The standards applied are as follows:

Visually assessed characters

Plots
Conventional varieties

Overall plot examination
Population Standard = 0.1%
Acceptance Probability = 95%
Equivalent to a maximum of 5/2000 off-types (variants) in the plots examined

Harvested sub-sample examination
Population Standard = 1%
Acceptance Probability = 95%
Equivalent to a maximum of 1/20 or 3/100 off-types (variants) in a representative sub-sample examination.

6.5 Resubmissions

For all varieties, except hybrid varieties, a resubmission of plant material may be allowed for a second growing cycle, if in the first growing cycle the number of plants did not exceed 15 plants in a sample size of 2000, (population standard of 0.5% with an acceptance probability ≥ 95%) or 9 plants or parts of plants in a sample size of 100, (population standard of 5% with an acceptance probability ≥ 95%). The period of reassessment for a resubmission is two years.
6.6 Hybrids

The products of hybridisation i.e. the F1 hybrid and the male sterile parent, are tested in plots as a population of plants since they are the product of obligate out-pollination. The following standard based upon TG/1/3 and standards applied in other Member States will be applied:

<table>
<thead>
<tr>
<th>Hybrid Type</th>
<th>Plots</th>
<th>Harvested ears</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 hybrid</td>
<td>10% @ 95% probability</td>
<td>10% @ 95% probability</td>
<td></td>
</tr>
<tr>
<td>CMS male sterile parent</td>
<td>0.2% @ 95% probability</td>
<td>2% @ 95% probability</td>
<td></td>
</tr>
</tbody>
</table>

The maximum permissible number of off-types in a seed plot of the hybrid will be 225/2000 and the maximum permissible in an examination of a representative sub-sample of harvested ears will be 16/100. The spring sown vernalisation test will consist of a single plot of approx. 600 plants and the maximum number of off-types will be 74/600.

The maximum permissible number of off-types in a seed plot of the CMS male sterile parent will be 9/2000 and the maximum permissible in an examination of a representative sub-sample of harvested ears will be 6/100. The spring sown vernalisation test will consist of a single plot of approx. 600 plants and the maximum number of off-types will be 4/600.

The pollinator and restorer lines are usually inbred lines and will be tested as conventional varieties using plots if entered for NL/PBR (see 6.4. above).

7 STABILITY

7.1 A variety is considered sufficiently stable when there is no evidence to indicate that it lacks uniformity or fails to conform to the essential characteristics of its description in different submissions or in different tests.

7.1.1 Hybrids

Hybrids may be considered to lack stability if there is evidence that their progenitor lines lack uniformity or fail to conform to the essential characteristics of their description.

7.1.2 For 3-way hybrids with segregating progenitor lines, the production and maintenance schemes of all progenitor lines must indicate that the final hybrid (candidate) can, in terms of its genetic constitution, be consistently reproduced in each cycle of propagation.

8 DUS REPORT AND VARIETY DESCRIPTION

8.1 Upon completion of the DUS examination the DUS Summary report will be submitted to APHA by the specified date. This report will specify all non-routine characteristics for establishing distinctness.

8.2 The final DUS report, including the full variety description, will be submitted to APHA by the specified date. The characteristics to be used in the description are identified in Section D.
APPENDIX 1

REFERENCE COLLECTION VARIETIES

1 NATIONAL LISTING

1.1 The DUS reference collection, for NL purposes, for any given category of plant variety comprises the following at the time when the application for the candidate is made:

1.1.1 All other candidate varieties already in DUS test in the UK, or entering testing at the same time as the candidate, including those being tested for other Member States.

1.1.2 All varieties on the UK National List including any entered for export only to another Member State.

1.1.3 Varieties on the EC Common Catalogue whose seed is known to be certified or marketed in the UK.

1.1.4 Varieties nominated by the authorities concerned where tests are done for other Member States.

1.1.5 Any varieties nominated by the applicant as being comparable i.e. known to be similar.

1.1.6 Any other varieties considered to be comparable i.e. known to be similar by the appropriate Test Centre or DUS Centre Group.

2 PLANT BREEDERS RIGHTS

2.1 The DUS reference collection, for PBR purposes, for any given category of plant variety comprises the following at the time when the application for the candidate is made:

2.1.1 All other candidate varieties already in DUS tests in the UK, or entering DUS testing at the same time as the candidate, including those being tested for other Member States or the Community Plant Variety office (CPVO).

2.1.2 Varieties protected in the UK, EC or in a UPOV Member State, which are known to be similar to the candidate variety.

2.1.3 Other available comparable varieties in common knowledge.