Animal & Plant Health Agency

United Kingdom National List Trials: Trial Procedures for Official Examination of Value for Cultivation and Use (VCU) Harvest 2019

White Clover

June 2019

Changes from Harvest 2018 VCU procedures

1. p8, C.4.2.1, Other Elements: sentence concerning sulphates added.

Contents

Section A General Information

A.1. Purpose

A.1.1 This document sets out the approved procedures to be used for growing trials, tests and assessments as required by the current **Protocol for Official Examination of Value for Cultivation and Use for White Clover**

A.2. Scope

A.2.1 These procedures apply to all varieties of White Clover.

A.3. Responsibilities

A.3.1 **Procedures Development Group**

The Procedures Development Group is responsible for reviewing these procedures annually and making amendments for which it has responsibility, in accordance with the provisions of the VCU Protocol.

A.3.2 **Trials Organisers and Operators**

A.3.2.1 Trials Organisers

a. England & Wales

British Society of Plant Breeders Ltd (BSPB)
BSPB House
114 Lancaster Way Business Park
Ely
Cambs
CB6 3NX

Tel No 01353 653846
Fax No 01353 661156
Email jeremy.widdowson@bspb.co.uk

b. Scotland

SASA
Roddinglaw Road
Edinburgh
EH12 9FJ

Tel No 0131 2448899
Fax No 0131 2448940
Email russell.thomson@sasa.gov.scot

A.3.2.2 The Trials Organisers are responsible for ensuring all **VCU Protocol** and **Procedures** requirements are followed and liaison with all Operators carrying out trials for National List purposes, including supply of seed and data handling.

3.2.3 Data Handling Operator

The Data Handling Operator identified by the Trials Organisers is responsible for trial design and data validation in accordance with the **VCU Protocol** and associated **Procedures.**

A.3.2.4 Growing Trial Operators, Seed Handling Operators and Quality Testing Operators

The Trials Organisers are responsible for proposing potential Growing Trial Operators and Quality Testing Operators to carry out trials and tests as determined by the Procedures Development annual review in accordance with the **VCU Protocol**, and these **Procedures**. The Trials Organisers are also responsible for finding Seed Handling Operators who are able to carry out seed handling. Seed Handling Operators prepare trial seed for sowing on behalf of any Growing Trial Operator in accordance with the **VCU Protocol** and these **Procedures**.

A.3.2.5 A list of all approved Organisers and Operators is shown in Appendix 1.

A.3.3 **VCU Protocol and Procedures non-compliance**

A.3.3.1 Where these procedures use the word “must” or “will” for any action then failure to carry out this action will result in non-compliance. Where the word “should” is used for any action then this is the method to be followed unless there are clear reasons not to, which can be justified by the operator as technically sound.

A.3.3.2 The Trials Organisers will forward any reports on **VCU Protocol or Procedures** non-compliance to APHA within 1 week of receipt. The Trials Organiser will obtain authorisation from APHA for any actions, including those necessary to remedy non-compliances, which are not within the requirements of the **VCU Protocol**. Such actions must be recorded as a non-compliance. Where emergency action is required and APHA staff are not available (e.g. evenings / weekends) the Trials Organiser should act but report this to APHA at the earliest opportunity. Where GMOs are concerned the arrangements are as detailed in section 3.4.

A.3.4 **Procedures for GM varieties**

A.3.4.1 The National Authorities and Trials Organisers will develop procedures for GM varieties if an application for a GM candidate variety is received.

A.3.5 **Handling of Trial Seed**

A.3.5.1 The Seed Handling Operator is responsible for organising the handling of seed of candidate varieties submitted by the applicant, and seed of control, or other reference varieties, in accordance with the requirements set out in these **Procedures** and the current **VCU Protocol**.

A.3.6 **Dispatch of Seed**

A.3.6.1 The Seed Handling Operator will arrange for seed to be dispatched by the agreed deadlines to the Growing Trial Operators, and, for authentication, to the DUS testing centres including, where appropriate, foreign testing authorities.

A.3.7 **Monitoring of VCU Growing Trial Operators and Seed Handling Operators – Documentation**

A.3.7.1 The appropriate Trials Organiser will take any necessary action to enforce deadline dates and quality standards for required documentation.

A.3.7.2 The Trials Organisers will ensure Growing Trial Operators and Seed Handling Operators have access to all current protocols and procedures relevant to them and that they are notified of any amendments.

A.3.8 **Seed Quantities**

A.3.8.1 The Trials Organisers will determine the quantity of seed required for all VCU tests and trials in each annual series, including authentication, and will notify the applicant of quantities and delivery addresses.

A.3 9 **Labelling of seed**

A.3.9.1 The Trials Organisers are responsible for ensuring all seed is clearly labelled with variety name/breeders reference and AFP number.

A.3.10 **Seed Quality**

A.3.10.1 Seed submitted for VCU testing must meet the standards for the final generation of seed given in the appropriate seed regulations, in respect of germination, analytical purity and content of other seeds and any other impurities.

A.4. Summary of Growing Trials, Tests and Assessments Procedures

A.4.1 The number of trials and site locations are as detailed in Appendix 4.

A.4.2 Control varieties are listed in Appendix 5.

A.4.3 The Trials Organisers are responsible for informing the Growing Trial Operators of the additional approved characters, which must be recorded as and when requested by applicants, and any samples that may be required for analysis.

A.4.4 **Special Tests**

An additional test for characters not specified in the procedures may be requested by the applicant. APHA is responsible for liaison with the Trials Organisers to produce a procedure for the conduct of a special test or trial. This procedure would require the approval of the National Authorities.

A.4.5 **VCU trial assessments required**

Bold = Obligatory *Italics = Additional if requested by the applicant*

White Clover

Type of Character	Reference	Description of assessment
Yield	Section C	**Dry matter yield of clover fraction in the second and third harvest years of the yield management.** **Dry matter yield of grass plus clover in the second and third harvest years of the yield management.**
Behaviour with respect to factors in the physical environment.	Section C	**Ground cover of clover in the spring and autumn of the persistence management**. **Ground cover of clover in autumn in the yield management.** **Resistance to winter damage.**
Seasonal dry matter yield	Section C5	**Yield of clover fraction in first cut, mid-season and last cut are measured in the second and third harvest years**
Resistance to harmful organisms	Section D	**Slugs (1-9 scale)** ***Sclerotinia* Disease (%)** **Pepper Spot (*Leptosphaerulina trifolii*) (%)** **Leaf Spot (*Pseudopeziza*) (%)** **Black Blotch (*Cymadothea trifolii*), (%)** **Downy Mildew (*Peronospora trifoliorum*) (%)** **Rust (*Uromyces*) (%)** **Sitona (%)**

A.4.5.1 **Further Measurements**

The following must be measured or recorded in all trials, following procedures in Section C.

Sowing Date
Clover density and Grass density (where there are plots in trial with poor establishment)
Harvest date
Pest Damage (where present at a level which will affect results)
Plot size

Section B Seed Handling Procedures

B.1. Responsibilities

B.1.1 The Seed Handling Operator or Growing Trial Operators are responsible for carrying out the following seed handling procedures.

B.2. Seed Handling Procedures

B.2.1 Seed Handling Operator/Growing Trial Operators will receive a sowing list from the Trials Organiser.

B.2.2 Seed Handling Operators/Growing Trial Operators must record receipt of seed from applicants by checking it against the sowing list as it arrives. APHA should be notified of any damage to the packaging, loss of seed or certification problems that would affect the validation of the trials.

B.2.3 The Seed Handling Operator must retain 20 grams of the seed submitted of every variety in the trial, for authentication by the DUS test centre.

B.2.4 Cross contamination must be avoided by ensuring equipment is clean between weighing and treatments.

B.2.5 Each seed handling operator must retain a 10 gram sample of seed until one month after the end of the trial.

B.3. Authentication of Seed Stocks

B.3.1 Year 1 VCU and DUS submissions are taken from the single submitted seed stock. Year 2 and any further VCU seed submissions are authenticated by the DUS Test Centre according to the procedures set out in the appropriate DUS Protocol, except when there is 1 single seed submission or submissions from the same seed lot.

B.3.2 All samples must be kept under suitable conditions for the authentication procedures required and must be clearly labelled and sealed.

B.3.3 The Seed Handling Operator must send requested samples to the DUS test centre by the date specified by APHA.

B.3.4 If the level of uniformity recorded in DUS tests is not uniform (COYU) or VCU authentication of a candidate the VCU tests will be considered invalid for that candidate in that season.

Section C Growing Trial Procedures

C.1. Responsibilities

C.1.1 The Growing Trial Operators are responsible for conducting the trials according to these procedures.

C.2. Site Suitability

C.2.1 The Growing Trial Operator will be responsible for providing a suitable site, which meets the following criteria:

C.2.2 Soil type should be typical of those on which white clover is grown locally. Soil fertility and texture should be uniform across the site. The soil should be sufficiently uniform to avoid variation in the growth of the trial.

C.2.3 Previous cropping must be appropriate for white clover crops to be grown.

C.2.4 The trial should be sited away from trees, hedges, headlands and other features, which are likely to cause uneven growth or encourage damage from pests.

C.2.5 The trial area should be cultivated in the direction of ploughing and drilled across the direction of ploughing and cultivation such that each plot receives similar wheeling compaction. Cultivations should follow best local practice.

C.3 Sowing the Trial

C.3.1 **Plot Size**

C.3.1.1 Plots must be drilled or broadcast to produce a minimum plot length of 4.5 m after cutting back. Minimum sown width is 0.9 m with a maximum unsown gap between plots of 0.5 m. Minimum harvest plot size is 6.5 m^2. The row number per plot should not be less than 10 rows for drilled plots. A buffer plot of minimum width of 0.5 m should be drilled between each trial plot. Two replicates will be sown.

C.3.2 **Plant population**

C.3.2.1 When drilling, self-cleaning type drills should be used to sow a mixture of perennial ryegrass and white clover at a seed rate of 3.5 kg/ha of white clover and 18 kg/ha of perennial ryegrass. Perennial ryegrass for use as a companion will be supplied by the testing co-ordinator. Sowing depth should be as would be appropriate for white clover. Care should be taken when sowing to maintain the homogeneity of the mixture along the plot. Alternatively, the clover and/or the grass can be broadcast over the plot area. Sowing rates used should be the same as for drilled plots.

C.3.3 **Trial layout**

C.3.3.1 The Trials Organisers following consultation with APHA produce provisional sowing lists. The Trials Organisers will make final sowing lists available to Growing Trial Operators, along with the trial plans produced by the Data Handling Operator.

C.3.3.2 The trial must be sown according to the plan produced by the Data Handling Operator and may be an incomplete block design. In an incomplete block design, each replicate is split into a number of sub-blocks. Any splitting of replicates must be between sub-blocks and not through sub-blocks. Varieties can be moved within a sub-block but must not be moved from their sub-block. If plots are moved out of their original sub-block, they will have to be treated as missing plots. The Trials Organiser must be informed immediately if there are any departures from the original plan or if there are any other anomalies. Botanically separated fractions should be analysed using a complete block configuration to avoid negative numbers being generated where very small fractions exist.

C.3.3.3 If there is a need to replace a planned variety e.g. if varieties are withdrawn, affected plots must be sown with any of the standard control varieties. Any such replacements must be agreed with the Trials Organiser. The control varieties are listed in Appendix 5.

C.3.4 **Sowing**

C.3.4.1 Care must be taken with drill settings and drilling speed to ensure satisfactory and uniform establishment and plant population from plot to plot. It is also important to ensure that there is no carry over of seed between plots. Growing Trial Operators should inform the appropriate Trials Organiser as soon as it is apparent that the establishment of any plot has been unsuccessful.

C.3.4.2 A discard plot of at least 0.5 m wide should be sown on either side of each trial plot to prevent clover stolons growing from one plot to the next. This discard plot should be maintained free of clover throughout the trial period by the use of appropriate approved herbicides.

C.3.4.3 Any missing rows or parts of rows or plot areas must be noted on the sowing plan and returned to the appropriate Trials Organiser so that a decision on the viability of these and adjacent plots can be made. It may sometimes be possible to patch in missing parts of rows without affecting the viability of the trial but this should only be done after consultation with the appropriate Trials Organiser if it is done after the sowing year.

C.3.5 **Confirmation of trial layout**

C.3.5.1 After the trial has been drilled, the Growing Trial Operator must:

a) Confirm that the trial has been drilled or broadcast according to plan and provide the sowing date, by returning site data 1 and associated trial sketch to the appropriate Data Handling Operator
b) If any amendments to the plan have been made, return a hard copy of the plan to the appropriate Data Handling Operator with any amendments clearly indicated. Alternatively, amendments may be notified electronically with the agreement of the Data Handling Operator.

C.4. Husbandry

C.4.1 **Agronomy**

Where not specified in these procedures agronomy should follow best local practice, advisory and regulatory guidelines. Application of fertilisers and sprays should be uniform. It is normally best to apply these across the direction of the plots. Application wheelings should not run through the harvested plot area.

C.4.2 **Fertiliser application**

Application of fertilisers should be uniform. It is normally best to apply these across the direction of the plots. It must take into account inherent fertility, previous cropping, winter rainfall and the best local practice. All fertiliser applications should take account of the AHDB Nutrient Management Guide (RB209), the corresponding advisory publications in England, Wales, Scotland and Northern Ireland and past trialling experience.

Details of fertiliser rates are given below:

Sowing year:

At the discretion of the Growing Trial Operator, but in line with official advisory publications (including RB209). Growing Trial Operators should note the necessity of adequate pH, phosphate and potash for clover establishment.

Harvest years 2 & 3

If the Growing Trial Operator considers that too little or too much clover is present in the first harvest year (where yield is not recorded) the rate or timing of the nitrogen application may be adjusted in order to attain a good clover content.

The aim in the yield management should be to maintain an average clover ground cover of 50% in the large leaf control (30% of the total dry matter yield as clover). Total nitrogen applications through the season should be subject to compliance with local advisory and regulatory guidelines.

As an example:

All harvest years:

Trial	Nitrogen as N	Phosphate as P_2O_5	Potash as K_2O
Yield management	40 kg/ha in February or March and a further 40 kg/ha after each of the first four cuts	Up to 175 kg/ha in spring depending on the soil requirements	Up to 175 kg/ha in spring and 175 kg/ha around the end of June, depending on the soil requirements
Persistence management	40 kg/ha in February or March, around the end of May and around the end of July	Up to 175 kg/ha in spring depending on the soil requirements	Up to 175 kg/ha in spring and 175kg/ha around the end of June, depending on the soil requirements

C.4.2.1 **Other Elements**

Sulphate should be applied along with nitrogen applications at a rate between 20 and 40% of the N rate. Thus, for a nitrogen application of 100 kg N /ha, sulphate would be applied at between 20 and 40 kg/ha, as SO_3. . In addition to the above lime should be applied at the discretion of the Growing Trial Operator and in compliance with official regional advisory publications and regulations. In the sowing year Growing Trial Operators should note the necessity of adequate pH, phosphate and potash for grass establishment.

C.4.3 **Herbicides**

Chemicals must not be used if there are any known varietal sensitivities. If in doubt the appropriate Trials Organiser should be consulted. Application should normally be across the direction of sowing.

C.4.4 **Growth Regulators**

These must not be used on white clover trials.

C.4.5 **Pest and Disease Control**

C.4.5.1 **Pest Control**

Sitona (pea and bean weevil) is the most likely insect pest. During the sowing year it should be controlled by appropriate means if necessary but treatment should not be done in the three harvest years unless the trial is jeopardised, but permission must first be sought from the appropriate Trials Organiser and reported to the Data Handling Operator.

Slugs can also damage the establishing trial and treatment with an approved molluscicide may be required in the sowing year. Treatment should not be done in the three harvest years unless the trial is jeopardised, but permission must first be sought from the appropriate Trials Organiser and reported to the Data Handling Operator.

If necessary, approved means should be used to prevent or minimise damage by field mice, birds and other vertebrate pests. Control should be carried out throughout the trial period and not just in the establishment year.

C.4.5.2 **Disease control**

Disease control should only be undertaken after agreement by the appropriate Trials Organiser.

C.4.6 **Irrigation**

Irrigation will only be permitted to facilitate establishment. Permission from the Trials Organiser is not required to do this.

C.4.7 **Pathways**

A gap (pathway) is required at the end of each plot to allow access for harvesting and fertiliser application. It is usual to sow the pathways with a dense slower growing grass for ease of maintenance and to allow machinery to travel in wetter conditions.

C.5 Harvesting

C.5.1 **Yield management**

C.5.1.1 **Sowing year**

Plots to be topped at the discretion of the Growing Trial Operator to produce a uniform clover content.

C.5.1.2 **First harvest year**

Cuts at a total grass plus clover yield of approximately 1500 kg/ha of dry matter (not weighed) at a cutting height of 30mm.

C.5.1.3 **Second and third harvest years**

Cuts at a total grass plus clover yield of approximately 1500 kg/ha of dry matter at a cutting height of 30mm. Yield recorded.

To prevent clover seed shedding in the plots it may be necessary to cut before the scheduled yield level is reached. If this is the case, a note should be attached to the data file explaining the situation.

C.5.1.4 **Excluded Harvests**

If there is insufficient growth to comply with the 3 or 4 week cutting cycles, the decision to apply fertiliser is the responsibility of the trials co-ordinator who has the option to omit a fertiliser application if this is consistent with best practice.

C.5.2 **Persistence management**

C.5.2.1 **Sowing year**

Plots to be topped at the discretion of the Growing Trial Operator to produce a uniform clover content.

C.5.2.2 **All harvest years**

Cutting to start when the sward is 60 mm high and cutting height as close to 20 mm as possible. Cutting should take place every 10 days until the end of June, then every 15 days until 15 November or until growth stops in the autumn. Harvested herbage to be removed

Trial to end after the autumn ground cover measurement is taken in the third harvest year.

C.5.3 **Harvesting method**:

C.5.3.1 **Yield trial**

Plots should be harvested using a specialist grass harvester with a reciprocating-knife cutter bar. The harvested herbage must be weighed either on-board or separately, using an electronic balance graduated to 0.1 kg. All harvested material must be removed from the plot after weighing.

Yield records should be transmitted electronically to the appropriate Data Handling Operator within seven working days of each cut.

C.5.3.2 **Persistence trial**

Use a rotary or cylinder mower to cut as close to 20 mm as possible. All harvested material to be removed from the plots without weighing.

C.5.4 **Samples –Yield Trial**

Sample collection for dry matter determination and botanical analysis is required in the second and third harvest years of the yield trial.

Either – where the botanical analysis will be done by hand separation it is necessary to take two representative samples from each plot at each harvest;- the first for dry matter determination, the second for botanical analysis.

Or – where it is proposed to use an approved NIR spectrometry method to determine clover content it is sufficient to use the dry matter sample from each plot once that sample has been dried and the dry weight recorded.

C.5.4.1 **Dry Matter determination – Oven Method**

A fully representative sub-sample of fresh material is accurately weighed, or an accurately recorded catch weight taken. The treatment of samples and the time interval between cutting and weighing should be such that there is no significant moisture loss between the weighing of the plot fresh yield and the weighing of the fresh weight of the sample. The fresh sample is recorded to the nearest 1.0 g.

If the plot fresh yield is over 300 g then the sample should be a minimum of 300 g. If the whole plot fresh yield is less than 100 g then the yield should be recorded as zero and no sample should be taken. If the whole plot fresh yield is between 100 g and 300 g then use the whole plot yield as the dry matter sample.

The samples are placed in the drier which must be at a temperature of 104 ^{o}C with the air recirculator set in the range 80-100% recirculation in order to restore the temperature to 104 ^{o}C as rapidly as possible. When the temperature is restored to 104 °C the air regulator is set at 80% recirculation i.e. 20% fresh hot air. The regulator is critical for rapid drying. The samples are dried at 104 °C for such time as is necessary for complete drying.

The dried sample is carefully removed from the drier as soon as the sample is cool enough for accurate weighing. The dry weight is recorded to the nearest 0.1 g.

When the dry weights are reported as a percentage, the fresh weight should be reported as 100.

C.5.4.2 **Botanical analysis to assess Clover Content.**

As outlined above this can be done in one of three ways:-

1) Hand separation of the second sample taken at each harvest, or
2) By NIR spectrometry on the oven dried sample following dry matter determination, or
3) By NIR spectrometry on board the harvester

Hand separation – second sample, minimum sample size 100g fresh weight.

The fresh sample should be physically separated into its component Clover and Grass (+/- weeds) fractions as soon as possible after each harvest. The separate fractions should then be oven dried and weighed to determine the Clover Portion Weight and the Grass Portion Weight so that the percentage Clover can be ascertained.

NIR Spectrometry – DM sample

Following oven drying and Dry Weight recording the sample should be milled and stored in labelled pots prior to despatch to:

NIAB Park Farm
Villa Road
Impington
Histon
CB4 9NZ Tel: 01223 233258

for approved NIRS analysis to determine Clover Content (% Clover).

On Board NIR Spectrometry

The NLSC is responsible for approving all equipment and calibrations. Prior to initial use of the calibration models and subsequently on an annual basis, a validation is carried out whereby a set of samples are analysed using the NIRS technique and the respective oven drying methodology (C.5.4.1). The results from the two techniques are analysed to ensure the accuracy of the NIRS calibration model.

C.5.5 **Submission of data**

C.5.5.1 Appendix 6 lists the records, with deadlines, to be sent to the Data Handling Operator. Diary sheets and other field records should be returned to the Trials Organisers immediately following the final cut of the season.

C.5.5.2 All plot records should be transmitted to the appropriate Data Handling Operator following the deadlines set out in Appendix 6. The Growing Trial Operators should ensure that data are free from errors before transmission. After scrutiny the results will be returned to the Growing Trial Operators for action as agreed by the appropriate Trials Organiser.

C.6 Records

C.6.1 There are four components:

1. **Diary** Field notes of trial status.

2. **Site data part 1** Site details; including site sketch, map and location, previous cropping, soil analysis fertiliser applications

3. **Site data part 2** Details of agrochemical applications and irrigation.

4. **Plot records** Plot data.

C.6.1.1 An entry in the Diary sheet should be made for any observations relevant to variety performance

C.6.2 **Plot records**

C.6.2.1 Plot data may be recorded direct onto a data logger or recorded on paper then entered and validated onto a computer using an approved system. A system of ensuring that data are recoverable, in the event of loss of original data, must be implemented, eg copy and safe storage. Whichever method is used, individual plot data will only be accepted at the appropriate Data Handling Operator in an approved format using the AFP number, variety name and units as listed in Sections C and D.

C.6.2.2 All observations should be checked at the time of recording to ensure that they lie within acceptable limits for the character recorded. Observations that have been designated as exceptional by the recorder should be identified with a note on the approved data file or hard copy medium describing the possible causes together with a recommendation for their exclusion or inclusion in the trial analysis.

C.6.2.3 Plot numbers on record sheets must correspond with the numbering on the field plan.

C.6.2.4 If a character is not recorded or is missing the Growing Trial Operator should indicate in the diary or on the recording sheet the reason why it has been excluded.

C.6.2.5 Where a plot record is missing the Growing Trial Operator should record this in any data file or hard copy medium as a symbol thereby indicating there is no recorded value associated with this plot.

C.6.2.6 Specific plot records should be made as counts or on the scales shown for each character. Only the character names as listed may be used.

C6.2.7 All records should be returned to the appropriate Data Handling Operator as soon as reasonably possible. Indicative deadlines are given in Appendix 6. All records must be returned by the final deadlines.

C.6.3 **Procedures for recording Characters**

The following procedures must be followed for measuring all characters to be used in NL decision-making.

C.6.3.1 **GROUND COVER** **(OBLIGATORY)** **(%)**

C.6.3.1.1 **Yield Management**

Record on the yield management seven to ten days after cutting in September or October of the sowing year and immediately after new leaves are fully expanded seven to 14 days after cutting in September or October in each of the three harvest years.

C.6.3.1.2 **Persistence Management**

Record, on the persistence management only, when new leaves are fully expanded seven to nine days after the second mowing has been made in the spring of each of the three harvest years. However, the record must be taken before 30 May so might have to be taken after the first cut in very late springs.

Additionally in September or October autumn ground cover is required for the sowing year and immediately after new clover leaves are fully expanded after cutting in September or October in each of the three harvest years. It may take 14 to 19 days for the new leaves to fully expand so delay cutting if necessary in order to make the record. Assess the ground cover of white clover (leaves, petioles and stolons) in each plot by eye either as % ground cover or on a one to nine scale where nine is most clover. Determine the percentage ground cover of the highest and lowest eye score within each replicate using a point quadrat, 100 points per plot first strike. Ignore any grass or weeds present in the plot. If preferred, it is permissible to quadrat every plot.

C.6.3.2 **FRESH YIELD** **(OBLIGATORY)** **(kg)**

Record at each cut of the yield management as given in Section C.5 of the Protocol. Enter the total harvested weight to the nearest 0.1kg in kg per plot and provide the harvested plot dimensions with the record. If the plot lengths or widths are not constant then these must also be entered as records using the character names PLOT LENGTH and PLOT WIDTH. Also, if there is a tare on the balance, this should be entered as TARE WT to the nearest 0.1kg

C.6.3.3 **DRY MATTER CONTENT** **(OBLIGATORY)** **(%)**

A detailed protocol for sampling for dry matter is given in Section C above.

C.6.3.4 **CLOVER PERCENTAGE OF TOTAL DRY MATTER WEIGHT**
(OBLIGATORY) (%)

Record at each cut in the second and third harvest years of the yield management to the protocol given in Section C5 above.

C.6.3.5 **GRASS PERCENTAGE OF TOTAL DRY MATTER WEIGHT**
(OBLIGATORY) (%)

Record at each cut in the second and third harvest years of the yield management to the protocol given in Section C5 above.

C.6.3.6 **SCLEROTINIA DISEASE** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.7 **PEPPER SPOT** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.8 **LEAF SPOT** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.9 **BLACK BLOTCH** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.10 **DOWNY MILDEW** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.11 **UROMYCES RUST** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.12 **SLUGS** **(OBLIGATORY if present)** **(1-9 scale)**

Slug damage can be severe on white clover especially in the spring or after wet weather. Record only if significant damage is seen on the most affected variety on the scale:

1 Most damage
9 No damage

Also record the approximate leaf area damaged on the most severely affected plot.

C.6.3.13 **SITONA** **(OBLIGATORY if present)** **(1-9 scale)**

Record only if significant leaf notching is seen on the most affected variety on the scale

1 Most damage
9 No damage

Also record the approximate leaf area damaged on the most severely affected plot.

C.6.3.14 **Site Factors**

Any factors which may have affected the yield of the trial or individual plots must be noted and accompany the yield data.
Records for other scores, including pests or diseases not specified in the procedures, may be recorded as plants affected on a 1 to 9 scale, and reported with definitions for each rating on the 1 to 9 scales.

C.6.3.16 **Trial Inspection**

All trials will be inspected by the Trial Inspection and Technical Validation Operator and, in some cases, it may be necessary to visit on more than one occasion.

The requirements of Growing Trial Operators in respect of inspections are to:

Give inspectors reasonable access to trials
Provide the inspector with information (for example pesticide sprays applied etc) at the time of inspection if requested.
Co-operate with the inspector in making any non-routine assessments required to establish the validity of the trial (for example population counts).
Carry out any action agreed in consultation with the inspector. In particular it is important that any requirement to shorten plots is undertaken and that missing values are returned for any plots excluded from the trial.

Section D Disease Testing Procedures

D.1 Assessment of Natural Infection

Recording of disease on the growing trials will be the responsibility of the Growing Trial Operator at the appropriate sites.

D.1.1 **Diseases Recorded**

D.1.1.1 No inoculated disease tests are carried out routinely.

D.1.1.2 No Disease Observation Plots are carried out routinely.

D.1.1.4 All disease assessments should be sent to the Data Handling Operator as soon as they are made.

D.1.2 **Naturally Occurring Disease in VCU Growing Trials**

D.1.2.1 Foliar disease on the clover component should be recorded when the level of infection on the most affected variety is over 5% of the leaf area. Percentage leaf area infected on the plot as a whole should be recorded using the key below as a guide.

D.1.2.2 *Sclerotinia* infection should be recorded using the instructions below as a guide. Examine plots from November to February at monthly intervals to detect the presence of apothecia of *Sclerotinia trifoliorum*. During February, March or April, record percentage of clover content killed by *Sclerotinia*. Clover killed by *Sclerotinia* will appear brown, and may have white mycelium present on the leaves. Bare patches will appear at the end of the winter and *sclerotia* (black “mouse-dropping” sized bodies) may be found. Since white clover is grown as a mixture with ryegrass, it is important to monitor crops closely, and associate clover death with the presence of apothecia before killed areas are replaced by ryegrass growth.

D.1.2.3 Other clover pathogens should be recorded when more than 5% of the plot area is affected. The percentage of the area infected in each plot should be recorded.

D.1.2.4 If disease infection persists, successive records should be made through the season.

D.1.2.5 White clover can be affected by a number of fungal pathogens which can affect yield, quality and re-growth. The most likely diseases to be encountered are clover rot (*Sclerotinia trifoliorum*), downy mildew (*Peronospora trifoliorum*), leaf spot (*Pseudopeziza*), black blotch (*Cymadothea trifolii*), rust (*Uromyces nerviphilus*) and pepper spot (*Leptosphaerulina trifolii*). The relative importance and most likely time of attack are given in the table:

	Importance	Time
Clover rot (*Sclerotinia trifoliorum*)	***	Winter
Pepper spot (*Leptosphaerulina trifolii*)	***	Summer onwards
Leaf Spot (*Pseudopeziza*)	***	Autumn
Black blotch (*Cymadothea trifolii*),	***	Summer and autumn
Downy mildew (*Peronospora trifoliorum*)	**	Spring
Rusts (*Uromyces nerviphilus*)	*	Summer

*** common and severe
** less common, can be severe
* infrequent.

D.1.2.6 Other clover diseases should be recorded if present at more than 5% of the leaf area (or 5% of plot area for other diseases) on the most affected variety and records sent to the Data Handling Operator. Confirmation of the identity of a disease should be obtained from an appropriate plant pathologist if required.

D.1.3 **Recording methods**

D.1.3.1 **Leaf diseases**

Instructions

1.	Examine all clover leaves in at least four areas of each plot.
2.	Include all necrosis and chlorosis attributable to disease to be assessed.
3.	Estimate % infection using the description below, interpolating values if necessary.
4.	Record the average % infection from the four areas.

Infection Disease Severity Description

0	No infection observed.
0.1	Older leaves with a trace of infection, other leaves uninfected.
1	Older leaves with up to 10% infection, other leaves largely uninfected.
5	Older leaves with up to 25% infection, middle aged leaves with a trace of infection.
10	Older and middle aged leaves with up to 25% infection, young leaves largely uninfected.
25	Leaves of all ages appear 50% infected 50% green on average.
50	Leaves of all ages appear more infected than green on average.
75	Very little green tissues left.
100	No green tissue left.

Section E Quality Testing Procedures

At present there are no Quality Tests for White Clover

Section F Trial Design and Data Handling Procedures

F.1 Plan Validation and Storage

F.1.1 After the trial has been sown, the Growing Trial Operator must:

a) Confirm that the trial has been drilled according to plan and provide the sowing date, by returning site data 1 and associated trial sketch to the appropriate Data Handling Operator.

b) If any amendments to the plan have been made, return a hard copy of the plan to the appropriate Data Handling Operator with any amendments clearly indicated. Alternatively, amendments may be notified electronically with the agreement of the Data Handling Operator.

F.1.2 The Data Handling Operator will check these for statistical validity and, once this has been done, will load the plan on the database.

F.2 Data Recording

F.2.1 Data are recorded using the methods and characters given in Sections C, D and E.

F.2.2 Site information is recorded for each trial including, for example, data on previous cropping, seed rates, soil details and fertiliser applications.

F.2.3 Details of any agrochemical applications are also recorded and retained by the Growing Trial Operator.

F.3 Other Tests and Trials

F.3.1 Any additional or alternative designs required for the assessment of additional VCU characters not detailed in Appendix 3 of the **VCU TRIAL PROTOCOL** for White Clover will be added to these **Procedures** as and when approved by the NLSC.

Appendix 1 Approved Trial Organisers/ Operators for White Clover

Activity	Organisers/Operators Responsible
Trial Design and Data Handling Operator	NIAB for England & Wales BioSS for Scotland AFBI for Northern Ireland
VCU Trials Organiser	BSPB for England, Wales & Northern Ireland BSPB & SASA for Scotland
Seed Handling Operator	NIAB
Growing Trial Operator	DLF, DSV UK and NIAB for England IBERS for Wales SRUC for Scotland SASA for Scotland AFBI for Northern Ireland
Pathology Trial Operator	NIAB
Trial Inspection Operator	NIAB and BSPB for England & Wales SASA and BSPB for Scotland AFBI and BSPB for Northern Ireland
Technical Validation Operator	NIAB for England & Wales BioSS for Scotland AFBI for Northern Ireland
Quality Testing Operator	NIAB
Data Review and Standard Setting Operator	NIAB

Appendix 2 Seed Treatment Products for Use on NL Trials

None

Appendix 3 Seed Dispatch Deadlines

VCU seed must be delivered to the Seed Handling Operator by 5th February.

Appendix 4 VCU Growing Trials

No of sowings	2
No of harvest years	second and third harvest years from each sowing. Persistency is recorded in all harvest years
No of trial sites	6
No of reps	4 – 2 persistence management + 2 yield management
Leaf size	all leaf sizes
No of control varieties	4 – 1 per leaf group
Trial regimes	Yield and persistency

Appendix 5 Control Varieties for VCU Assessments

Small Leaf - AberAce
Medium Leaf - AberDai
Large Leaf - Alice
Very Large Leaf Aran

Companion Grass – AberMagic

Leaf Size Groups in White Clover

The applicant must allocate varieties to the correct leaf size group. These are classified as follows:

Small	Smaller than Crusader.
Medium	Equal to or larger than Crusader but equal to or smaller than Merwi.
Large	Larger than Merwi but smaller than Triffid.
Very Large	Equal to or larger than Triffid.

unit = terminal leaflet length x breadth (mm^2)

Appendix 6 Dates for Submission of Records

Dates by which Records should be sent to Data Handling Operator

Record	Latest date of receipt
Site data part 1 (including site sketch)	Within 2 weeks of sowing the trial
Site data part 2	Annually by end of November
Yield records	Electronically to the appropriate Data Handling Operator within seven working days of each cut.
Plot records (in approved electronic format)	Annually by end of November

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Any enquiries regarding this publication should be sent to us at

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The Animal and Plant Health Agency (APHA) is an executive agency of the Department for Environment, Food & Rural Affairs, and also works on behalf of the Scottish Government and Welsh Government.