Animal & Plant Health Agency

United Kingdom Variety List Trials: Trial Procedures for Official Examination of Value for Cultivation and Use (VCU) Harvest 2022

Red Clover and Lucerne

Reviewed April 2022

Changes from Harvest 2021 VCU procedures

1. p1, A.3.2.1 – updated contact details
2. p24, Appendix 5, Control Varieties – Diane removed from lucerne controls.

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 Red Clover and Lucerne

A.2.Scope

A.2.1 These procedures apply to all varieties of Red Clover and Lucerne.

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 Trial Organisers

a. England and Wales

British Society of Plant Breeders Ltd (BSPB)
BSPB House
114 Lancaster Way Business Park
Ely
Cambs. Jeremy M: 07747 567351
CB6 3NX Louise M: 07917 046705
Email jeremy.widdowson@bspb.co.uk

b. Scotland

SASA
Roddinglaw Road
Edinburgh Tel No 0131 2448899
EH12 9FJ Fax No 0131 2448940
Email russell.thomson@sasa.gov.scot

A.3.2.2 The Trials Organiser is 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.

A.3.2.3 Data Handling Operator

The Data Handling Operator identified by the Trials Organiser 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 Organiser is responsible for proposing potential Growing Trial Operators and Quality Testing Operators to carry out trials and tests as determined by the Procedures Development Group annual review in accordance with the VCU Protocol, and these Procedures. The Trials Organiser is 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 Organiser 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 Organiser 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 Trials Organiser 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. The Trials Organiser will ensure that any seed treatments or additives are fit for the purpose. Seed treatment products are listed in Appendix 2.

A.3.6 Dispatch of seed

A.3.6.1 The Trials Organiser 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 Trials Organiser will take any necessary action to enforce deadline dates and quality standards for required documentation.

A.3.7.2 The Trials Organiser 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 Organiser 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 Organiser is 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 Organiser is responsible for informing the Growing Trial Operators of the additional 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*

Red Clover and lucerne

Type of character	Reference	Description of assessment
Yield	Section C	**Dry matter yield under conservation management in the first, and second harvest years (lucerne) and in the first, second and third harvest years (red clover).**
Behaviour with respect to factors in the physical environment.	Section C	**Ground cover in autumn of each harvest year** **Resistance to winter damage**
Seasonal dry matter yield	Section C5	*First, second, third and fourth cut conservation yields are measured in each of the harvest years.*
Resistance to harmful organisms	Section D	**Sclerotinia trifoliorum, stem rot (%) (red clover only)** **Stem eelworm (%)** **Downy mildew (%)** **Verticillium wilt % (Lucerne only)** **Slugs (1-9 scale)** **Sitona (1-9 scale))**
Quality characteristics	Section E	**Crude protein, second cut, second and third harvest years**

A.4.5.1 **Further measurements**

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

Sowing date
Establishment weakness
Harvest date
Pest damage (where present at a level which will affect results)
Plot size
Ground cover in sowing year

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 The Seed Handling Operator/Growing Trial Operators will receive a sowing list from the Trials Organiser, along with instructions as to which seed treatments or additives may be used. The chemicals approved by the Procedures Development Group are listed in Appendix 2.

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/Growing Trial Operator must retain 20 g 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 g 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.

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 APHA will select samples from Growing Trial Operators/Seed Handling Operators for authentication at DUS test centre.

B.3.4 Seed Handling Operators must send requested samples to the DUS test centre by the date specified by APHA.

B.3.5 Where there is more than one Seed Handling Operator, APHA will select samples for authentication from at least two Seed Handling Operators.

B.3.6 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 the 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 red clover and lucerne are 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 red clover and lucerne 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 wild fauna.

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. Three replicates will be sown.

C.3.2 **Plant population**

C.3.2.1 When sowing, Self-cleaning type drills should be used to sow at the following seed rates:

Red clover
Candidates 13 kg/ha

Lucerne (should be sown before end of May to ensure establishment, seed should be inoculated prior to sowing)

Candidates 20 kg/ha

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.

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 sowing speed to ensure uniform distribution of seed in each plot. It is also important to ensure that there is no carry over of seed between plots. Trials 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 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 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.

Examples of fertiliser rates are given below:

Sowing year:
At the discretion of the Growing Trial Operators, but in line with official advisory publications (including RB209).

First, second and third harvest years:

Spring phosphate	up to 175 kg/ha P_2O_5 depending on the soil requirements
Spring and July potash	up to 175 kg/ha K_2O in spring depending on the soil requirements and up to175 kg/ha in July depending on the soil requirements.
Sulphur	up to 40 kg/ha of SO_3 in spring depending on the soil requirements and up to 40 kg/ha in July depending on soil requirements.

In addition to the above lime etc should be applied at the discretion of the appropriate Growing Trial Operators. In the sowing year, Growing Trial Operators should note the necessity of adequate pH, phosphate and potash for establishment.

C.4.3 **Herbicides**

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

C.4.4 **Growth regulators**

These should not be used on red clover and lucerne trials.

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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 this must be reported to the Growing Trials Organiser.

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 this must be reported to the Growing Trials Organiser.

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 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 **Red clover**

C.5.1.1 **Sowing year**

Plots to be topped at 60 mm at the discretion of the Growing Trial Operator without weighing to produce a uniform dense sward by the end of the season.

C.5.1.2 **First, second and third harvest year**

Up to four cuts per year. Cutting height as close as possible to 60mm.

The cutting dates to use are as follows

First cut	early flowering or earlier if lodged
Second cut	six weeks after the first cut.
Third cut	six weeks after the second cut.
Fourth cut	mid October.

C.5.1.3 **Excluded harvests**

If a scheduled harvest in any management is omitted due to low yields (not more than 300 g of fresh material on any plot), then all the plots are fertilized as specified in these procedures.

C.5.2 **Lucerne**

C.5.2.1 **Sowing year**

Plots to be topped at 60 mm at the discretion of the Growing Trial Operator without weighing to produce a uniform dense sward by the end of the season.

C.5.2.2 **All harvest years**

Cutting heights as close as possible to 60 mm

First cut	early flowering or earlier if lodged
Second cut	six weeks after the first cut.
Third cut	six weeks after the second cut.
Fourth cut	mid October.

C.5.2.3 **Excluded harvests**

If there is insufficient growth (ie less than an estimated 300 g fresh material on any plot) to comply with the 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.5.3. **Harvesting method:**

C.5.3.1 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.5.4 **Samples**

C.5.4.1 A representative sample should be taken from each plot at each cut and dried to assess total dry matter yield.

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 °C with the air recirculator set in the range 80-100% recirculation in order to restore the temperature to 104 °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 for such time as is necessary for complete drying.

The dried sample is carefully removed from the drier and 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 Samples, for protein content testing, should be forwarded immediately to:

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

It is important that samples are despatched promptly after harvest. Notification of sample dispatch should be faxed to the appropriate Trials Organiser at the same time.

C.5.5 **Submission of data**

C.5.5.1 All records should be transmitted to the 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 Trials Organisers.

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 and 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 using a system approved by the Trials Organiser 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, e.g. copy and safe storage. Whichever method is used, individual plot data will only be accepted at the appropriate Data Handling Centre 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 identified 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.

C.6.2.7 All records should be returned 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)** **(%)**

Record in the autumn of the sowing year and once in autumn in each harvest year.

When scoring red clover/lucerne ground cover, assess the ground cover of sown species in each plot by eye either as % ground cover or on a one to nine scale where nine is most clover/lucerne. 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 weeds present in the plot. If preferred, it is permissible to quadrat every plot.

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

Enter the total harvested weight to the nearest 0.1 kg 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 0.1 kg.

C.6.3.3 **DRY SAMPLE WEIGHT** **(OBLIGATORY)** **(g)**

A detailed protocol for sampling for dry matter is given in Section C.5. Also specify the fresh weight taken for the sample (FRESH SAMPLE WT, FRESH WT). If the figures are DM% then enter the fresh weight of sample as 100.

C.6.3.4 **SCLEROTINIA** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.5 **DOWNY MILDEW** **(OBLIGATORY if present)** **(%)**

Record as described in Section D

C.6.3.6 **SLUGS** **(OBLIGATORY if present)** **(1-9)**

Slug damage can be severe on red 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.6.3.7 **STEM EELWORM** **(OBLIGATORY if present)** **(1-9)**

Record if present, estimate percentage of plants affected.

C.6.3.8 **VERTICILLIUM WILT** **(OBLIGATORY if present)** **(%)**

Record only if present when levels of damage are greater than 5% of plants in the worst plots. Estimate percentage of plants affected.

C.6.3.9 **SITONA** **(OBLIGATORY if present)** **(1-9)**

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.6.3.11 **Establishment weakness**

Growing Trial Operators must monitor all newly sown trials for sward establishment. Poor establishment in any plot or trial, that may affect variety performance should be reported to the Trials Organiser, who may request ground cover scores (0-9) to be recorded. Such incidences should be reported in the same way as for non-compliances and all other test sites alerted to ensure that it is not seed lot related.

C.6.3.10 **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 should be taken as plants affected on a 1 to 9 scale. Include definitions for each rating on the 1 to 9 scales.

C.6.3.11 **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:

1. Give inspectors reasonable access to trials
2. Provide the inspector with information (for example pesticide sprays applied etc) at the time of inspection if requested.
3. Co-operate with the inspector in making any non-routine assessments required to establish the validity of the trial (for example population counts).
4. 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 Red clover/lucerne 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.1.2.3 If disease infection persists, successive records should be made through the season.

Disease	Time
Stem rot (*Sclerotinia trifoliorum*)	Winter
Downy mildew (*Peronospora trifoliorum*)	Spring

D.1.2.4 **Leaf diseases**

Instructions

1.	Examine all clover / Lucerne 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

E.1 Responsibilities

E.1.1 The Quality Testing Operator appointed by the Trials Organiser is responsible for conducting the approved quality tests according to these procedures.

E.2 Quality assessment methodology for obligatory and additional tests

E.2.1 Samples are collected for dry matter and protein analysis as indicated in Section C. Although in some instances all of the sampling and weighing of fresh material may be carried out in the field, it is acceptable for samples to be brought to the laboratory for weighing. If the latter option is followed the representative sample is immediately sealed in a 500-gauge polythene bag and kept out of direct sunlight and as cool as possible until transported to the laboratory. Each sample is identified with a label.

E.2.2 Dried material from the following cuts should be retained for protein analysis. Instructions for milling these samples are given below. Samples from each replicate should be bulked for each variety and milled following oven drying. Samples to be despatched to the Testing Co-ordinator for analysis:

Red clover

Cut 2 in the second and third harvest years.

Lucerne

Cut 1 in the first harvest year.

E.2.2 Quality tests

E.2.2.1 **Milling of dried samples for further quality analysis (see Section C.5.4)**

1. The dry matter samples from both replicate plots must be combined and a representative sample taken for milling (sufficient to provide 150 ml of milled material for analysis).
2. The mill must be a hammer mill fitted with a screen with 1.0mm apertures. Screens must be checked for wear of the inside surface at regular intervals. Frequent use causes the circular 1.0 mm hole to elongate, and when the elongation reaches 1.2 mm the screen must be changed.
3. Samples for milling must be absolutely dry. This can be achieved either by milling immediately after weighing out of the dryer or by re-heating dried samples to 104 °C for 1 hour before milling.
4. The mill must be thoroughly clean before use.
5. The mill must be at maximum speed before the sample is introduced gradually to prevent the mill labouring.
6. All of the sample must be removed from the receptacle and thoroughly mixed. Care must be taken at all stages to prevent the loss of fine powder which is a critical part of the milled sample.
7. After mixing, a representative sub-sample should be taken in the following manner:
   a) If less than 150 ml of milled sample, all of it should be placed in the sample tubs.
   b) If more than 150 ml of milled sample, the tub should be filled with a fully representative sub-sample that has been fully mixed before placing in the tub.
8. The sample tub must be sealed with a close fitting lid and labelled with information in an approved format.
9. The milled samples must be sent to the laboratory for analysis immediately immediately and by 15 September at the latest, with appropriate identification documentation.

E.2.2.2 **Crude protein analysis**

This is evaluated by the Quality Testing Operator using “Dumas Gas Analysis”.

Section F – Trial design and data handling procedures

F.1 Plan validation and storage

F.1.1 After the trial has been drilled, 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 red clover and lucerne will be added to these Procedures as and when approved by the NLSC.

Appendix 1 – Approved Trial Organisers/ Operators for red clover and lucerne

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 SASA for Scotland
Growing Trial Operator	DLF Seeds Ltd, DSV UK and NIAB for England IBERS for Wales SRUC for Scotland AFBI for Northern Ireland
Seed Handling Operator	NIAB
Pathology Trial Operator	N/A
Trial Inspection Operator	NIAB and BSPB for England & Wales SASA + BSPB for Scotland AFBI + 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

Rhizobium meliloti inoculum for lucerne

Appendix 3 – Seed dispatch deadlines

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

Appendix 4 - VCU Growing trials

Minimum number of sowing years:	2 for lucerne, and red clover
Number of harvest years	2 harvest years for first sowing and second sowing – Lucerne 3 harvest years for the first sowing and two harvest years for the second sowing – red clover
Number of trial sites:	3 for red clover 1 for lucerne
Number of replicates	3 in each trial
Ploidy groups	Diploid and tetraploid varieties of each group are grown in the same trial but candidates are compared with the appropriate control variety. See Appendix 5
Trial regimes	As detailed in Section C.5. Maximum 5 cuts in sowing year. Conservation management in first and second harvest years (and third harvest year – red clover).
Number of control varieties	One for each ploidy

Appendix 5 – Control varieties

Red clover:

Amos (Tetraploid control)
Merviot (Diploid control)

Lucerne:

Marshall

Appendix 6 - Dates for submission of records and samples

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

To Quality Testing Operator

Quality testing – samples to be coarse milled	To Quality Testing Operator by 15 September
Quality testing – samples to be fine milled	To Quality Testing Operator by 15 September

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www.gov.uk/apha

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.