

PROTOCOL FOR THE EXAMINATION OF
VALUE FOR CULTIVATION AND USE OF
FORAGE GRASSES AND WHITE CLOVER
USED FOR GRAZING AND CUTTING

2017

Raad voor plantenrassen (Rvp)
Plant Variety Board

Commissie Samenstelling Aanbevelende Rassenlijst (CSAR)
Recommended List Committee

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1. Introduction

This protocol sets out the procedures to be used for the examination of the Value for Cultivation and Use (VCU) of forage grass varieties and white clover in the Netherlands.

After a minimum period of trials (see 2.4), the *Raad voor plantenrassen* (Plant Variety Board) decides whether or not the variety can be included in the National List based on the VCU results.

Varieties included in the National List are approved for marketing.

After one year of additional testing (see 2.4) the *Commissie Samenstelling Aanbevelende Rassenlijst* (Recommended List Committee) decides whether or not the variety can be included and classified in the Recommended List.

2. General

2.1 Principles of examination

This protocol is based on the assumption of sufficient basic knowledge of the cultivation, management and husbandry of pasture and treatment of trials. The agronomy of the trials should follow the best local practice of an average Dutch cattle farm. Commonly used methods, treatments and techniques are not explicitly described, unless different crop management is a requirement of the protocol.

VCU testing with the aim of assessing and establishing the main characteristics of new varieties is performed on trials. Varieties included in the Recommended List are also sown as standards.

This protocol describes the VCU tests for forage grass varieties and white clover aimed at obtaining sufficient data of varieties to be included in the National List and the Recommended List.

2.2 Admission to VCU

All new applications of varieties of forage grasses and white clover can be admitted to the VCU tests on condition that a timely application for registration is submitted/has been submitted in one of the EU member states. This applies to application for inclusion in the National List. For varieties to be included in the Recommended List, the industry has designated a number of institutes where testing for registration must take place. For more information, refer to the protocol Decisions on Inclusion, Classification, Order and Deletion from the Recommended List of Varieties of Forage Perennial Ryegrass

No pre-trial results are necessary and no limit on the number of varieties to be tested applies.

Applications for new varieties for VCU testing must be submitted to the Plant Variety Board and the Trials Organiser before 15 January using an application form (see Appendix 1).

2.3 Start of examination

A VCU testing cycle is started every year for new varieties of late and medium heading perennial ryegrass. A VCU testing cycle is only started for new varieties of the (minor) grassland species early heading perennial ryegrass, Italian ryegrass, hybrid ryegrass, timothy, meadow fescue and white clover when there are sufficient numbers of applications per species. Sufficient is understood to mean at least four new varieties per minor species. Plantum NL makes an inventory of the number of applications of minor species in January of each year, and indicates when a new VCU testing cycle of minor species can start.

2.4 Duration of examination

Trials of varieties of late and medium heading perennial ryegrass registered for VCU testing are sown in two consecutive years. A trial for cutting and a trial for grazing is sown each year. The four trials are sown in different regions.

The cutting trials for early heading perennial ryegrass, Italian ryegrass, hybrid ryegrass, timothy and meadow fescue are normally sown in two consecutive years.

See Appendix 3 for an overview of the trial plans.

The table below shows the number of years before a decision is made on including the variety in the National List or in the Recommended List as well as the total duration of the trials. The period of time before the decision is made is calculated from the first moment of sowing. The first year of sowing is therefore also included.

Crop	Decision on inclusion in National List after a minimum of	Decision on inclusion in Recommended List after a minimum of	Duration of complete trial
Late and medium heading perennial ryegrass	3 years	4 years	5 years
Early heading perennial ryegrass	3 years	4 years	5 years
Italian and Hybrid ryegrass	2 years	3 years	3 years
Timothy and Meadow fescue	3 years	4 years	5 years
White clover	3 years	4 years	5 years

3. Varieties to be tested

3.1 New varieties

All new applications can be included in the trials. At the end of each trial year the breeder or the representative of the variety can decide which varieties continue in the trials. Premature termination of the testing of a variety does not entitle the applicant to a refund of the application fee for the entire examination.

3.2 Recommended List varieties (late and medium perennial ryegrass)

As well as the new varieties in the trial, the VCU also includes the majority of the A (generally recommended) and N (newly recommended) varieties of the Recommended List as reference or standard varieties.

The range of Recommended List varieties of late and medium heading perennial ryegrass is categorised into permanent, rotating and new reference varieties. The total number of reference varieties sown (permanent, rotating and newly Recommended List varieties) is limited to 8-10 varieties per heading type.

The testing of these varieties can also be considered as a retest or control test. The results are included in the figures for the Recommended List.

The final choice of reference varieties is established by the Technical Committee of Plantum in agreement with CSAR and the Plant Variety Board.

Permanent reference varieties

Two diploid and two tetraploid A-varieties are used as permanent reference varieties in trials of late and medium heading perennial ryegrass.

The best classified A-varieties of the latest Recommended List are generally designated as permanent reference varieties. The range of permanent reference varieties can therefore change each year. However this is limited to a maximum of half the number of permanent reference varieties. Other Recommended List varieties can be designated as permanent reference varieties after consultation, if this is considered necessary to obtain better variety comparison. Plantum should be informed of this in time by the Trial Organiser. The permanent reference varieties are sown on all trials of the heading type concerned.

Rotating reference varieties

All the other A- and N- varieties (i.e. not the B-varieties, which are soon becoming outclassed) not included in the trials as permanent reference varieties are considered rotating varieties. These rotating reference varieties are sown every two years on at least one grazing or cutting trial (late and medium heading perennial ryegrass).

New reference varieties

The newly recommended varieties are also sown in two consecutive years in a grazing or cutting trial immediately after the decision on inclusion on the Recommended List was made.

Long-term standard varieties

A minimum of two long-term standard varieties are included in the trials of late and medium heading perennial ryegrass. One early heading long-term standard variety is included in the cutting trials of medium heading perennial ryegrass (diploid and tetraploid alternately). The Trial Organiser designates which long-term standard varieties are used. This variety is sown in both the late and medium heading trial. When designating the long-term standard variety, preferably use the standard variety with a heading date that is as close as possible to the heading date of the other group.

3.3 Recommended List varieties (early perennial ryegrass, timothy, meadow fescue, clover, Italian ryegrass and hybrid ryegrass)

Four good reference varieties are included per trial with early heading perennial ryegrass, Italian ryegrass, timothy and white clover, three with meadow fescue and two with hybrid ryegrass. The reference varieties do not need to be identical in both sowing years of a testing cycle but should be identical for at least half the number of reference varieties. The Trial Organiser also designates which reference varieties are used in this instance.

4. Trial Seed

Appendix 1 shows an overview of the standards of the seed samples of new and reference (Recommended List) varieties in terms of quantity, quality, submission date, submission address etc.

4.1 New varieties

The seed of newly applied varieties must be breeder's seed. The applicant, or representative, must submit the amount of seed of each variety to be tested to the Trials Organiser on time. A seed sample is only requested when a testing cycle starts of the species or heading type concerned. The required amount is requested as one batch and will be used to sow all trials in both consecutive years of testing.

4.2 Reference varieties

Seed used for the reference range (of Recommended List varieties) must originate from a certified seed lot. A copy of the certificate must be supplied to the Trials Organiser. The seed is supplied by the variety representative. The lot number concerned must be stated.

The Trials Organiser is responsible for requesting the seed.

If, for valid reasons, seed is not submitted for testing, it is possible to suspend testing for a maximum of one year.

5. Trial schemes

A description follows of the trial schemes for the individual species and heading types. See Appendix 2 and Appendix 3 for overviews of the trial systems and schemes for the various species (or heading types). These overviews may be adapted and updated in consultation by the Trials Organiser each year.

The cutting trials of the minor species should be combined as far as possible with the cutting trials of late and medium heading perennial ryegrass. This may implicate that the first cut, in particular, of the minor species (see 6.2 for Italian and hybrid ryegrass) may sometimes be cut at a slightly later (heavier) stage.

5.1a Late and medium heading perennial ryegrass

The varieties are sown in one grazing trial and one cutting trial in both the first and second year. The grazing trials are sown on sandy soil. The cutting trials are sown on sandy soil and clay soil. The trials are sown in spring. The trials are left intact for four years including the year of sowing. Yield determination takes place in the 2nd, 3rd and 4th year. After four years (before a decision is made regarding inclusion in the Recommended List) five sets of yield data for grazing trials and five for cutting trials have been obtained.

5.1b Early heading perennial ryegrass, applied for as medium heading

All the extra costs relating to conversion of a variety from medium to early heading will be charged to the applicant(s). These costs are additional to the costs for application and testing in the medium heading group.

5.2 Early heading perennial ryegrass

Varieties of early heading perennial ryegrass are sown in a cutting trial in both the first and second year, if possible combined with medium heading perennial ryegrass.

The first cut of all varieties in the trial is made at the moment the medium heading varieties are ready to be cut.

The trials are sown on sandy and clay soil and are left intact for four years including the year of sowing. Yield determination takes place in the 2nd, 3rd and 4th year.

After four years, five sets of yield data have been obtained.

5.3 Italian ryegrass and hybrid ryegrass

The varieties are sown in two trials in the first year: spring sowing and autumn sowing. One trial is sown on sandy soil and one on clay soil.

The choice of whether sand or clay soil is sown on first depends on the sowing of the cutting trial for late and medium heading perennial ryegrass. The yield is determined for the spring sown trial on clay soil (or sandy soil) in the year of sowing and the following year. The yield is determined for the autumn sown trial on sandy soil (or clay soil) in the two years following the year of sowing.

In the second, or possibly third, year a trial on sandy or clay soil is sown in autumn. The yield is determined for this trial in the year after the year of sowing only.

In this way, after three, or four, years, the yield has been determined a total of five times.

5.4 Timothy and meadow fescue

The timothy and meadow fescue varieties are sown in one cutting trial and one competitor trial in the first year. The cutting trial is combined with the cutting trials of late and medium heading perennial ryegrass sown on clay soil.

The cutting trials are left intact for four years including the year of sowing. Yield determination takes place in the 2nd, 3rd and 4th year. After four years, the yield has been determined a total of three times.

The competitor trials are sown on sandy soil and are left intact for four years including the year of sowing. Observations are made for four years.

5.5 White clover

The varieties of white clover are sown in spring and autumn in the first year both in one grazing trial and one competitor trial.

The grazing trials are sown on sandy soil and are left intact for three years including the year of sowing. Yield determination takes place in the 2nd and 3rd year. After three years, four sets of yield data have been obtained.

The competitor trials are sown on clay or sandy soil and are left intact for four years including the year of sowing.

6. Trials

The varieties of forage grasses and white clover are, depending on the species and heading type, sown in grazing trials, cutting trials, competitor trials and if necessary on special trials (identity or row plots). Alongside the new varieties, Recommended List varieties are also included in the trials as permanent and rotating reference varieties. The important characteristics are observed and assessed on the trials. Yield determination is only conducted on the grazing and cutting trials.

6.1 Grazing Trials

Only late and medium heading varieties of perennial ryegrass and white clover are sown on the grazing trials. The perennial ryegrass varieties are sown as monoculture. The white clover varieties are not sown on these trials as monoculture, but in a mixture with perennial ryegrass (BG 3).

The minimum plot size is 32 m² (4 x 8 m).

The trial is mainly used for grazing, with a few cuts taken. The total number of cuts per year is five to seven. Three to four are general cuts under grazing conditions and two to three are cuts used for silage. The cuts taken for silage must not normally be made in immediate succession. Cattle (dairy) must be used to graze the trial.

To determine the fresh yield, a strip of 4 to 5 m² from the approx. 32 m² plot is cut just before the cattle are put out to pasture. Each subsequent cut is taken from the patch adjacent to the previous cut. After four or five cuts, the next cut is taken from the same patch as the first cut. This stipulation may be deviated from if the strip that is due to be cut is not representative. After the yield determination, the trial must be grazed bare over a period of two to four days. Immediately after the grazing period, the strip designated for the following yield determination, and possibly the entire field, is topped over to remove grazing residuals.

6.2 Cutting Trials

The varieties of all forage grasses are tested on cutting trials. White clover is not sown in cutting trials. The forage grass varieties are sown as monoculture. The minimum plot size is 9 m² (1.5 x 6 m).

The trial is used for cutting only. The number of cuts of the perennial varieties, with the exception of Italian ryegrass and hybrid ryegrass, is five to six. The number of cuts of Italian ryegrass and hybrid ryegrass, is six to seven in the course of a full trial year. Due to the different growth rate, the first two cuts of Italian ryegrass/hybrid ryegrass are not taken at the same time as the other species. From the third cut onwards, providing the crop yield permits, these species will be cut at the same time as the second and subsequent cuts of medium and late heading perennial ryegrass. The other species will generally be cut more or less at the same time.

To determine the yield, a plot area of approx. 7.5 - 9 m² is cut. The entire plot can be used or the area required can be cut from a larger plot. If only part of the plot is cut, the remaining uncut areas of the plot must be cut on the same day.

6.3 Examination of competitive species in Competitor trial

Competitor trials are sown to examine timothy, meadow fescue and white clover varieties in mixtures with perennial ryegrass. The varieties are sown as monoculture and as a mixture with perennial ryegrass (BG 3 = permanent meadow mixture).

The minimum plot size is 21 m² (3 x 7 m). Each plot is divided into two sub-plots. The first sub-plot (approx. 9 m²) is left as monoculture of the variety sown; the other sub-plot (also containing the variety sown) is overseeded with perennial ryegrass (BG 3). The seed rate for timothy and meadow fescue is 15 kg/ha and for white clover 25 kg/ha. The trials must be managed in an identical way to the grazing trials; i.e. mainly used for grazing. The main characteristic assessed in these trials is the competitive capacity of the varieties.

6.4 Special trials (authentication trials or row plots)

In order to verify identity / varietal trueness, the VCU samples of the new varieties are compared with the identity samples (S-samples) submitted for DUS testing. The samples are sown pairwise in row plots for this purpose. In most cases, these trials can be combined with the existing row plots for DUS testing. If a variety has not been applied for in the Netherlands, the seed must be requested from other DUS testing authorities. (See Annex 5).

7. Trial layout and trial management

7.1 Trial field

Only fields that are regular and uniform in soil fertility and texture are considered suitable for use as trials. No trials must have been performed on these fields in the previous two years. In the case of drained trial fields, the trial lanes must run parallel to the drains and the plots must lie across the direction of the drains. Treatments such as ploughing and seedbed preparation and crop cultivation at a later stage, must be performed parallel to the trial lanes creating similar wheeling compaction on each plot.

Enough space must be left around the trials to exclude the influence of headlands and to allow for machinery to be turned.

A trial can be ended prematurely due to irregular or poor emergence. If possible, a replacement trial should be sown as soon as possible. If an on-going trial has to be ended due to irregularities that occur at a later stage, the decision to end the trial, and whether or not the data gathered up until that point is valid, is taken in consultation (between the Trial Organiser, the VCU Group of Forage Grasses, CSAR and the Plant Variety Board). Statistical analysis forms the basis for this decision.

7.2 Trial plan

Trials are sown according to the trial plan drafted by the Trials Organiser. In principle, the trials of perennial ryegrass consist of three complete replicates (blocks), except for the grazing trial that consists of four replicates and the row plots that usually consists of two or three replicates. Trials of timothy, meadow fescue, Italian and hybrid ryegrass are sown in four replicates.

Within a replicate, the plots are randomised. If eight or more varieties are tested, a replicate (block) is split into sub-blocks of four to seven plots. The sub-block composition should be well-balanced.

The trial plan is then a balanced incomplete block design.

The varieties of each replicate should preferably be grown in a single lane or strip. If this is deviated from, due to the actual dimensions of the trial or field, the boundaries of the sub-blocks must be taken into account. Any splitting of replicates must be between sub-blocks and not through sub-blocks. A lane therefore always comprises complete sub-blocks. Space must be provided in the trial plan for discard plots at the front and at the end of the lanes.

If the authentication trial / row plot (VCU samples + DUS samples) is combined with the DUS test, this trial will be the responsibility of the DUS testing organisation. In this case, the trial will be sown and managed by the organisation in charge.

7.3 Sowing

The spring sown trials must be sown in the period from early March until mid-May. The grazing trial on clay soil can also be sown at the end of the summer. Autumn sowing of Italian ryegrass must be done in the period from end August to mid-October. In years with extreme weather conditions, this planning can be deviated from to ensure a successful establishment of a new trial.

Trials must be sown mechanically with a special plot seeder. This ensures that the plots are sown in the right dimensions, and avoids the carry-over of seed of different varieties. The required row distance is 8-9 cm with single-sown rows. With so-called wide fluted coulter, a larger distance can be used.

The quantities of seed to be sown of the various species are stated in appendix 4.

7.4 Management, fertilising and husbandry

Management and husbandry of the trials should follow the best local practice of an average (good) Dutch cattle farm in managing pastures. The agronomy should follow the best local practice.

However, priority is given to identical treatment of all the plots within a trial.

The trials must be fertilised in compliance with common pasture practice.

The Dutch Fertiliser Advisory Program (BAP) is used as a guideline.

Chemical weed control treatment may be applied if too many weeds, e.g. chickweed (*Stellaria media*), appear at crop emergence. Chemical weed control is only permitted in later years to safeguard the validity of a trial. The presence of couch grass (*Elytrigia repens*) in trials must be avoided by preventive control of this weed prior to sowing the trial.

The trials may be irrigated. Irrigation should follow local practice. A good overlap must be taken into consideration.

8. Observations and measurements

8.1 Characteristics

The following characteristics must be observed in the VCU of Forage Grasses and White Clover. All observations must be reported.

The determination of the yield is discussed in chapter 9.

Emergence/establishment

Assessment of speed of emergence/density of seedlings a few weeks after sowing. Observation rated on a scale of 1-9 (9 = fast).

Ground cover (persistence) / sward density

The ground cover is the percentage of the sown variety that has established in the plot.

Assessment is performed twice a year: at the end of summer (August/September) and at the end of the growing season (October/November). Observation rated as a percentage.

Winter hardiness

Any winter damage is assessed visually by examining the extent of damage of plants/sward of the sown variety. Any simultaneous infection by snow mould (*Fusarium nivale*) must be assessed independently as far as possible. To obtain a sound assessment, it may be necessary to repeat the observation after one or two weeks to properly assess the actual extent of damage. Observation rated on a scale of 1-9 (1 = severe damage).

Extent of heading

This observation is only performed in the second and third (possibly fourth) cut. With Italian ryegrass, this characteristic is also assessed in the year of sowing (spring sowing). Observation rated as a percentage.

Competitive capacity

On the competitor trials, the competitive capacity in a mixture with perennial ryegrass is assessed by observing the percentage of ground cover of the variety sown. The monoculture is assessed for persistence. Assessment is performed at the beginning of the growing season (February/March), at the end of summer (August/September) and at the end of the growing season (October/November). Observation rated as a percentage.

Disease resistance

Any diseases that occur, such as crown rust (ryegrasses), leaf spot diseases (various grasses) or *fusarium* (mainly perennial ryegrass) are visually observed. Observation rated on a scale of 1-9 (9 = no infection). To obtain a sound assessment, it may be necessary to repeat the observation after approx. one week, to properly establish the progress of infection and to assess the varieties as well as possible.

8.2 Data recording

The Trials Organiser is responsible for performing the necessary observations and measurements. Certain activities, such as harvesting the trials can be contracted out, to regional applied research stations for example. In this case the provisions in the protocol apply in full force. The Trials Organiser, the applied research station and possibly the Trial Operator must confirm the nature and extent of the activities in writing.

Characteristics should be scored based as far as possible on the actual differences. On each trial, and in each year, the characteristic should be rated on an identical scale as far as possible. A high score is generally given in case of a favourable (positive) assessment of the characteristic, and a low score in case of a negative assessment of the characteristic.

The observations are recorded in writing or digitally saved. They are processed and summarised by the Trials Organiser at the end of the growing season, or earlier. The method of supplying, storing and processing the data is established by the Trials Organiser in a protocol. All yields and observations, including multi-year overviews, must be released to the applicants before 1 January.

Breeding Companies with varieties being tested in the trials may visit the trial sites. For these visits the protocol of the Trials Organiser on visiting VCU trials of forage grasses applies (see appendix 8).

9. Determination of the yield

To determine the dry matter yield, the fresh (green) yield from all the cuts on the grazing and cutting trials is measured and the dry matter content is determined.

Harvesting the fresh yields should preferably be done using a Haldrup harvester.

9.1 Time of harvest

On the grazing trials, a cut is harvested at the moment that approx. 90 % of the late and medium heading perennial ryegrass varieties have a dry matter yield of 1.5 - 1.8 tonnes per hectare.

A silage cut is harvested at a dry matter yield of 3.0 - 4.0 tonnes per hectare. On the cutting trials, harvesting is done at that moment that approx. 90 % of the late and medium heading perennial ryegrass varieties have a dry matter yield of 3 - 4 tonnes per hectare; for Italian ryegrass and hybrid ryegrass this is 3 - 4 tonnes per hectare in the first two cuts. Cuts taken in the autumn can be slightly lighter for both cutting and grazing trials.

Varieties of the same heading type or species on a trial must be harvested in a single action. If, due to adverse weather conditions during harvesting, it proves to be impossible to harvest a trial in a single action then at least the complete replicate (block) that is being harvested at the time must be harvested. The remaining part of the trial should be harvested as soon as possible.

9.2 Yield determination and sampling

For the determination of the fresh yield, the required area of each plot (grazing trials 4-5 m²; cutting trials 7,5 - 9 m²) is cut using the Haldrup harvester and weighed. The cutting height for cuts in grazing trials is approx. 5-6 cm; for cuts in cutting trials 6-8 cm. With a heavy cut, the longest cutting height applies.

A representative sample is taken from the cut fresh material. This sample is used to determine the dry matter content. Sampling must be done as soon as possible after the fresh yield has been weighed.

An ideal sample weighs 300 g of fresh material. Sampling can be done mechanically (by equipment on-board the Haldrup) or manually using a manual probe. To prevent losses caused by plant respiration, the samples must be processed as quickly as possible. If logistics prevent this, the samples must be kept in cold storage at a temperature of 4-6 °C. To prevent moisture loss, the samples must be kept out of direct sunlight on the field. The sample weighing approx. 300 g is dried in its entirety.

9.3 Determination of dry matter content

Unperforated or perforated plastic bags are used to hold the samples. Depending on which type of bag is used, follow the instructions below:

Unperforated plastic sample bag

Place the sample in the bag. Press the bag to remove all the air. Firmly close the bag by knotting the top. Store the sample as cool as possible. To dry a sample of fresh material remove it from the bag and place it in a drying tin (stainless steel or aluminium). Weigh the tin and contents. The measurement recorded can include the weight of the tin. The tin weight is excluded as a tare weight. Now dry the sample at 103 °C, until a constant weight is reached. If the sample has to be stored for NIRS purposes, the sample must be dried at a maximum of 60 °C until a constant weight is reached. Drying usually requires 18-24 hours, depending on the moisture content and the drying capacity: for NIRS samples 48 hours. After the drying period, the dry samples are weighed again as soon as possible.

Perforated plastic sample bag

Place the sample in a perforated plastic bag (able to withstand a minimum of 105 °C). On the trial field, collect the sample bags and place them in one, large, plastic bag. Firmly close the full bag used to collect the samples by tying the top. Store this bag underneath a pile of grass during further harvest activities. Start pre-drying the samples as soon as possible. Weigh the fresh sample in the perforated bag. Pre-dry the sample in a perforated (plastic) container at 50 °C for 24 hours. Now dry the sample at 103 °C, until a constant weight is reached. If the sample has to be stored for NIRS purposes, the sample must be dried at a maximum of 60 °C until a constant weight is reached. Drying usually requires 18-24 hours, depending on the moisture content and the drying capacity: for NIRS samples 48 hours. After the drying period, the dry samples are weighed again as soon as possible.

NIRS determination

All dry matter samples must be made available to Plantum so they can be used for further NIRS analysis. Plantum will indicate (at least 3 months in advance) which samples should be saved. Plantum will request the samples for further analysis within 12 months after harvest.

NIRS online.

If the dry matter content is determined using a NIRS online system, 10% of the samples must be taken as a control sample of which the dry matter content must be determined by drying.

10. Calculation of final scores

Over all characteristics that are to be observed according to the protocol, the average score is calculated using a dataset of at least the last three and maximum the last 10 calendar years during which the yield was determined.

The calculation of the final scores (moving multi-year average) aims to cover a period of maximum 10 years during which a characteristic was assessed.

An exception to this is winter hardiness. As soon as significant differences are observed, varieties can be rated with a score based on less than the stated minimum of three calendar years (in consultation with the Technical Committee, the Recommended List Committee and the Plant Variety Board).

11. Log

The Trials Organiser has a logbook. This logbook is used to record all treatments, as well as abnormalities and unexpected aspects that may influence the results of the trials. The logbook states information including sowing date, harvest dates of various cuts, treatments (grazing or cutting cut), fertilisation, husbandry such as weed control and irrigation, cutting and sampling errors, abnormalities on trial fields (urine burn, molehills), etc.

If the trials are managed on various sites (applied research stations), a logbook is present per site. The logbook should be made available to interested parties.

12. Quality assurance

Procedures and protocols relating to quality assurance are present at the Trials Organiser (or will be established). These documents establish and describe the further details of performing VCU testing. The VCU procedures and protocols should be made available to parties requesting VCU testing. Quality assurance is performed by the Technical Committee for Forage Grasses at Plantum. Final responsibility for approval and use of the data for the purpose of the National List and the Recommended List rests with the Plant Variety Board and CSAR respectively.

Appendix 1

Seed samples of new applications and reference varieties

1. New applications

The breeder/representative of varieties for new applications must submit a seed sample for VCU testing themselves. The amount is requested once and is to be used for sowing/testing of two sowing years.

Amount of seed to be submitted in kg:

Late and medium heading diploid perennial ryegrass	3
Late and medium heading tetraploid perennial ryegrass	4
Early heading diploid perennial ryegrass	2
Early heading tetraploid perennial ryegrass	3
Hybrid ryegrass diploid	3
Hybrid ryegrass tetraploid	4
Italian ryegrass diploid	3
Italian ryegrass tetraploid	4
Timothy	1.5
Meadow fescue	2
White clover	1.2

Seed used for new varieties must be breeder's seed

Remarks: Submitting seed of varieties for new applications is only necessary if a testing cycle of the heading type/species concerned is started. This is every year for late and medium heading perennial ryegrass. This does not apply to other species. The frequency depends on when a testing cycle starts.

2. Reference varieties (Recommended List varieties)

The A- and N-varieties from the Recommended List are sown as reference varieties. The Trials Organiser compiles a list of the reference varieties concerned and the amount of seed required. This list is sent to the breeders or representatives in early December. The seed sample is submitted by the breeder or the breeder's representative free of charge. The sample must consist of seed from a certified lot destined for the domestic market. The lot number must be stated.

3. Submission date

The Trials Organiser must receive the seed samples no later than 1 February.

4. Address for submission:

Proefbedrijf Praktijkonderzoek AGV
(Experimental farm of Wageningen University - Applied Research dept. PPO-AGV)
Edelhertweg 1
8219 PH Lelystad, NL

N.B: Applications for listing and/or PBR for new varieties to be tested must be submitted to the Plant Variety Board and the Trials Organiser before 15 January. The front and back of the TQ (Technical Questionnaire) for VCU Forage Grasses and White Clover must also be completed and sent to the Plant Variety Board and the Trials Organiser (see www.raadvoorplantenrassen.nl)

Appendix 2

Trial system of forage grasses and white clover

1. Late and medium heading perennial ryegrass

- Two consecutive sowing years
 - 1st year: 1 cutting trial sand
 - 1 grazing trial sand
 - 2nd year: 1 cutting trial clay
 - 1 grazing trial sand
- spring sowing
- order of sand or clay soil may be chosen
- trial duration including year of sowing 4 years; yield determination in year 2, 3 and 4.

2. Early heading perennial ryegrass, (in cycle)

- Two consecutive sowing years
 - 1st year 1 cutting trial sand/clay
 - 2nd year 1 cutting trial sand/clay
- spring sowing
- trial duration of cutting trials including year of sowing 4 years; yield determination in year 2, 3 and 4
- testing frequency depending on number of applications

3. Italian ryegrass/Hybrid ryegrass (in cycle)

- Two consecutive sowing years
 - 1nd year 1 cutting trial spring sowing sand/clay
 - 1 cutting trial autumn sowing sand/clay
 - 2nd year 1 cutting trial autumn sowing sand/clay
- yield determination 1st trial (first year spring sowing): in year 1 and 2
- trial duration including year of sowing 2 years
- yield determination 2nd trial (first year autumn sowing): in year 2 and 3
- trial duration including year of sowing 3 years
- yield determination 3rd trial (second year autumn sowing): in year 3
- trial duration including year of sowing 2 years
- testing frequency depending on number of applications

4. Timothy and meadow fescue (in cycle)

- One year of sowing with 1 cutting trial on clay and 1 competitor trial on sand
- spring sowing
- trial duration cutting trials including year of sowing 4 years; yield determination in year 2, 3 and 4
- trial duration competitor trials 4 years
- testing frequency depending on number of applications

5. White clover (in cycle)

- In the first year:
 - spring sowing: 1 grazing trial clay
 - 1 competitor trial clay or sand
 - autumn sowing: 1 grazing trial sand
 - 1 competitor trial sand or clay
- trial duration grazing trials including year of sowing 3 years; yield determination in year 2 and 3
- trial duration competitor trials 4 years
- testing frequency depending on number of applications

Appendix 3

Trial scheme of forage grasses and white clover

(+ = year of observation and/or year of harvest)

1. Late and medium heading perennial ryegrass

	Year 1	Year 2	Year 3	Year 4	Year 5
<u>Grazing trials</u>					
Site 1	sowing	+	+	+	
Site 3		sowing	+	+	+
<u>Cutting trials</u>					
Site 2	sowing	+	+	+	
Site 4		sowing	+	+	+

2. Early heading perennial ryegrass, (medium heading in cutting trials)

	Year 1	Year 2	Year 3	Year 4	Year 5
<u>Cutting trials</u>					
Site 1	sowing	+	+	+	
Site 2		sowing	+	+	+

3. Italian ryegrass and hybrid ryegrass

	Year 1	Year 2	Year 3
<u>Cutting trials</u>			
Spring sowing	sowing and harvest sowing	harvest	
Autumn sowing		harvest	
Autumn sowing		sowing	harvest

4. Timothy and meadow fescue

	Year 1	Year 2	Year 3	Year 4
<u>Cutting trials</u>				
(clay) Site 1	sowing	+	+	+
<u>Competitor trials (sand)</u>				
Site 2	sowing	+	+	+

5. White clover

	Year 1 Spring	Year 1 Autumn	Year 2	Year 3	Year 4
<u>Grazing trials</u>					
Site 1	sowing		+	+	
Site 2		sowing	+	+	
<u>Competitor trials</u>					
Site 3 (or 1)	sowing		+	+	+
Site 4 (or 2)		sowing	+	+	+

Appendix 4

Amount of seed to be sown in the trials

The amounts of seed of the various species stated below must be sown in the trials in normal conditions. Any poorer quality seed of a particular variety is not taken into account, or compensated for. Amounts in kg per ha.

<u>Sown as monoculture</u>	<u>kg/ha</u>
Perennial ryegrass diploid	45
Perennial ryegrass tetraploid	50
Hybrid ryegrass diploid	45
Hybrid ryegrass tetraploid	50
Italian ryegrass diploid	45
Italian ryegrass tetraploid	50
Timothy	25
Meadow fescue	40
White clover (amount in mixture with perennial ryegrass)	10

Overseeding perennial ryegrass (BG 3) in competitor trials:

Timothy and meadow fescue	15
White clover	25

Appendix 5: Determination of heading date

Determination of heading date of varieties that have been applied for Listing or for Plant Breeders' Rights in another EU member state than the Netherlands

Perennial ryegrass varieties are classified in maturity groups in the Recommended List according to the heading date. The heading date is determined in the single plant trials of the DUS test performed by the Variety Testing department of Naktuinbouw. As a consequence the heading date (in Dutch conditions) is unknown for varieties that have not been tested in the Dutch DUS trials. Requesting heading dates from foreign institutes is not always possible, neither is the correct comparison with standard varieties to determine the heading date. The heading date is a fixed date for maturity grouping.

Application

It is possible to indicate on the TQ (Technical Questionnaire) for VCU testing whether the new application concerns a variety that has already undergone DUS testing in another EU member state than the Netherlands, or whether these tests are taking place. The DUS testing must take / have taken place at an institute recognised by the VCU Decision Protocol for the Recommended List.

Performing testing of heading date

Planning

Submit application using the TQ-VCU to the Plant Variety Board and Livestock Research. Livestock Research informs Naktuinbouw of the list of names before 1 February. Submit seed to PPO before 15 February.

Amount of seed

No extra seed required

Trial layout

According to Technical Guideline for DUS testing based on single plant trials of the DUS testing department of Naktuinbouw in Tollebeek (at the Experimental farm of the Trial and Seed Inspection Agency NAK)
Sowing in three consecutive years in April.

Observations

Heading date in the year after sowing

Reporting

Naktuinbouw informs Livestock Research of the heading date of the varieties concerned annually in January. Information on the dates of other varieties being tested and the Recommended List varieties is also provided.
Livestock Research calculates the standardised heading date.

Appendix 6

Authentication of standard sample / VCU sample

Performed by Naktuinbouw, DUS Testing dept., Roelofarendsveen

Authentication procedures

- Planning (deadlines):
 - 1 March seed lists at Naktuinbouw
 - 1 May seed submission at Naktuinbouw
- Amount of seed
 - All species: 50 g
- Trial design
 - One plot consists of 2 rows of 2.5 m
 - Two replicates
- Trial Site
 - Tollebeek (Experimental farm of NAK)
- Sowing
 - End May/ early June
 - Sowing for authentication purposes and VCU will run synchronously, so that any results of the authentication tests can be used at the second sowing of VCU trials.
- Observations

	Naktuinbouw
Year of sowing	
Foliage colour	X
Width of foliage	X
Growth habit	X
Year after sowing	
Heading date	X

- Reporting

1. Naktuinbouw informs Trials Organiser of any suspect cases as soon as the observation is made.
2. Trials Organiser informs breeders.
3. Naktuinbouw, Trials Organiser and breeder establish the abnormality on site.
4. Breeder studies origin of material and approves/rejects the established abnormalities.
5. If breeder rejects the findings, electrophoresis/field testing will take place at Naktuinbouw. Costs will be charged to breeder.
6. Electrophoresis is performed on the two samples using a standard enzyme system. The result of electrophoresis is binding.
7. The two samples are examined and measured on site as in single plant trials. The result of the examination is binding.

Appendix 7

Sowing and planning

To be completed annually by the Trials Organiser.

Appendix 8

Protocol regarding on-site visits to VCU trials.

Independent visits to the variety trials (grazing and cutting trials) used for VCU Forage Grass by grass breeders and stakeholders is permitted subject to the following conditions:

1. Stakeholders at breeding companies (and representatives of breeders) of varieties that are being tested for VCU Forage Grasses, or with reference varieties taking part in trials for inclusion in the Recommended List are permitted to visit the trial site.
2. Visits by breeding companies to the trials must be announced at least two days in advance to the Trial Operator and/or applied research station concerned. The Trials Organiser is informed by e-mail.
3. The appointment with the Trial Operator and/or applied research station to visit the trials can only be made by a permanent, designated person (contact person) at the company concerned. This person is usually the (forage) grass breeder. The Trial Operator and/or applied research station are aware of the name(s) of the contact person(s).
4. The list of names/addresses/telephone numbers of Trial Operators and/or applied research stations is updated annually. A list is also made of details of the contact person(s) at the breeding companies.
5. The Trial Operator or applied research station decides whether a visit to a trial may proceed or not. If a trial is being grazed (cattle on the field) at the time of the visit, this particular trial cannot be visited. Fences and barriers cannot be changed. Other trials run by the same Trial Operator can be visited.
6. The permitted maximum number of visitors in a group, including the contact person at the breeding company, is four. Visits must take place during the daytime, in either a morning or afternoon slot, to avoid any disruption at the applied research station.
7. Larger groups can visit the trials under supervision of the Trials Organiser. This also applies if the visitors are primarily interested in the design and methods used in the variety trials.
8. If it is only possible to visit a trial site by crossing a farmyard at the Trial Operator/applied research station, entering the barn is not permitted.
9. In the event of an outbreak of contagious animal diseases or if other sanitary measures have been implemented, entering the farmyard and pastures (including the variety trials) at the Trial Operator or at the applied research station is not permitted. The breeding companies must be informed of this matter as soon as possible.
10. Trial sketches or trial designs are sent to the breeding companies as soon as the labelling, pathways etc. of a new trial have been laid out. This is approx. half way through the year of sowing. The trial design will be given to the contact person only. Further distribution of this information is not permitted.
11. Removing any plant material from the trial is strictly forbidden at all times.

Appendix 9

Contact details

Commissie Samenstelling Aanbevelende Rassenlijst (CSAR)
Recommended List Committee

Contact: David Kasse
Tel: + 31 (0) 793 030 333 / +31 (0) 6 52 06 43 26

Visitors address:
Louis Braillelaan 80
2719 EK Zoetermeer, NL

kasse@bo-akkerbouw.nl
www.bo-akkerbouw.nl
www.rassenlijst.info

Wageningen Livestock Research (Trials Organiser)
Project CGO Grasland
Postbus 338
6700 AH Wageningen, NL

Visitors address:
De Elst 1
6708 WD Wageningen, NL

Contact: Jan-Rinze van der Schoot
Tel: +31 (0)320 291 359

janrinze.vanderschoot@wur.nl
www.ppo.wur.nl

Raad voor Plantenrassen (Rvp) / Naktuinbouw
Plant Variety Board / Naktuinbouw
Postbus 40
2370 AA Roelofarendsveen, NL

Visitors address:
Edelhertweg 1
8219 PH Lelystad, NL

Contact: Lubbert van den Brink
Tel: + 31 (0) 6 10 96 09 17

L.vd.brink@naktuinbouw.nl
www.naktuinbouw.nl
www.raadvoorplantenrassen.nl

NAK
Seed Inspection Service of Arable Crops
Postbus 1115
8300 BC Emmeloord, NL

Visitors address:
Proef- en Controlebedrijf
Oortwijn Botjes Hoeve
Johannes Postweg 1
8309 PE Tollebeek, NL

wkooij@nak.nl