

Guide to the Work Protocol form

Fred Poelma

Table of Contents

I.	Working the new WP form version 7.0: Pilot	3
II.	General information – study dossier and work protocol.....	4
III.	Routing WP – filling out and evaluating the work protocol.....	6
IV.	Work Protocol - Cover page.....	8
V.	Explanatory notes of the questions	11
	1. Description of the experiment and statistics	11
	1.a. Abstract. Also give a short summary in simple Dutch.....	11
	1.b. What is the specific research question in this work protocol and how does this fit in the research strategy?.....	11
	1.c. Experimental design and statistics	11
	1.d. Is this a randomised experiment?.....	11
	1.e. Is this a blinded experiment with respect to animal treatment and analysis of the results experimental groups?	11
	2. Animals.....	12
	d. Surplus animals	13
	3. Housing.....	14
	a. Will antibiotics and/or other anti-microbial prophylactics and/or therapeutics be administered?	16
	b. Will anaesthesia be administered?	16
	c. Will analgesics be administered?	16
	5. Describe the consecutive procedures.....	17
	6. Welfare check and humane endpoint.....	18
	7. Estimated maximum discomfort by experimental group	20
	8. Ill and dead animals during the experiment	20
	9. Destiny of animals at the end of the experiment	20
VI.	Guide – Hazardous Substances Overview	21
VII.	Guide to registration with NVWA	22

I. Working the new WP form version 7.0: Pilot

The form is still protected, but no longer with a password. This protection remains necessary to enable completion of drop-down menus and check boxes. To add images and/or extra rows in tables, you can (temporarily) remove the protection as follows:

- Click on the “review” tab.
- Click on the “restrict editing” button.
- Click on “stop protection” at the bottom of the side bar that is now visible.
- You can now add images and/or extra rows.
- To add one (or more) extra row(s) to the table, select the row under which you want to add the extra row and copy/paste [Ctrl C]/[Ctrl V] until you have added the desired number of rows.
- Switch the protection on again with the button in the right sidebar. Do not enter a password!
- If you copied row with content, replace the text in the added rows with new text.

II. General information – study dossier and work protocol

1. The researcher must gather all the relevant information at the level of the study in a study dossier format. This is to improve the overall organisation and thus to enable better quality control and review (for example, by the responsible researcher him/herself, the IvD/AWB Utrecht (on behalf of establishment licence holders UU and UMC Utrecht) and/or NVWA inspector).
2. The study dossier (including the work protocol (WP)) must be kept close to the animals concerned for the duration of the experiment, where it can be available to employees of the animal facility, researchers, IvD Utrecht employees, NVWA inspectors and ISO/AAALAC auditors, etc.
3. The Experiments on Animals Act ('Wod' in Dutch) requires the responsible researcher to:
 - a. keep the relevant data (including the original of the work protocol and the records and results) of experiments on animals available for inspection for at least 3 years;
 - b. keep the documentation of a project that must be subjected to retrospective evaluation (such as cases of severe discomfort) at least until this evaluation has been completed.

A study dossier consists of parts 1 through 11 below:

1. chronological list of changes to the work protocol (Overview changes WP)
2. the individual changes to the WP
3. approved work protocol (WP)
4. if applicable, RIE's (Risk Inventory and Evaluation) and other addenda.

Procedures related to observation and monitoring welfare, documented on the following forms:

5. the welfare log¹
6. individual housing (optional; GDL and RMI have a fixed format)²
7. unexpected discomfort report and follow-up (optional; GDL and RMI have a fixed format)²

Procedures to be performed on laboratory animals (other than welfare-related action), documented on the following form:

8. procedures list (and completed procedures)³
9. work instructions from researcher to laboratory animal facility (optional)
10. instructions (optional)
11. other correspondence

¹ In the event of additional study-specific observations, the researcher is free to supply a form **in addition to** the **welfare log**, if the welfare log is insufficiently appropriate to document the additional study-specific observations.

² The GDL and RMI use a **welfare log**, a **form for documenting individual housing** and a **procedures list**. The GDL and RMI also have a procedure and form for **reporting unexpected discomfort**. The establishment licensees require **every** laboratory animal facility to use these documents (welfare log, individual housing form, procedures list) **or a comparable format better fitting the type of research/laboratory animal/laboratory animal facility**.

³ The WP and **procedures list** are closely related. The WP contains an overall planning and a description of the animal experiment (table 5.b. planning experimental procedures). The procedures list is a day-by-day procedural plan devised by the researcher using the planning of experimental procedures. The procedures list is used to note the times of planned experimental procedures (other than welfare monitoring) **before** performing them, and is also used **during** the experiment to record a particular procedure once it has been performed on an animal or group of animals. It goes without saying that the procedures list must correspond to the planning as listed in table 5.b of the WP, 'planning experimental procedures'. If the current format procedures list is not sufficiently appropriate, the researcher can submit a format more appropriate to the study than the GDL format.

Researchers and laboratory animal facility employees who are actively involved in the study in question must keep track of their procedures and correspondence in one study dossier. They use the appropriate forms **together**. Thus the laboratory animal facility employee and the researcher **do not** each keep their own separate **welfare log**; there is only one welfare log, in which both the laboratory animal facility employee and the researcher notate their own findings separately. This also applies to other forms such as those involving **individual housing and the procedures list**.

Any **changes** to the **work protocol** can be reported through the **Modification form**. See also www.ivd-utrecht.nl.

The following forms + guides can be found on the website of the IvD/AWB-Utrecht (www.ivd-utrecht.nl):

- Work protocol
- Work protocol breeding without discomfort
- Work protocol breeding with discomfort
- Sample Procedures list
- Welfare evaluation form
- Guide to the Work Protocol form
- Guide to the experiment design and statistics
- Modification form
- Route for submitting changes to research projects

III. Routing WP – filling out and evaluating the work protocol

The Study Director (SD, verantwoordelijke uitvoerende in Dutch) starts the process of evaluation and approval of the WP:

Study Director (SD)

- 1) The Study Director (SD) named in the work protocol can be the Principal Investigator (PI, verantwoordelijke onderzoeker in Dutch) named in the project licence application) or another researcher.
- 2) The SD downloads the most recent version of the WP + guide, and the procedures list (See General information) from the website of the IvD/AWB (www.ivd-utrecht.nl).
- 3) The SD sends by e-mail the draft WP, the procedures list(s), and any applicable attachment(s) to the IvD/AWB (info@ivd-utrecht.nl). Do not send more than one WP (including attachments) at one time.

IvD

- 4) The IvD/AWB checks the draft WP for to see if all the attachments (if applicable) and procedures list(s) have been included.
- 5) The IvD/AWB dossier holder evaluates who to involve in evaluating the WP (IvD dossier holder, veterinarian, laboratory animal facility contact person⁴, etc.), depending on the nature of the WP.
- 6) The IvD/AWB asks those who will be involved in the evaluation (scientist, statistician, etc.) to indicate within 1 day if they are available to participate in the evaluation. The IvD/AWB sends an e-mail to the laboratory animal facility's contact person and others involved in the evaluation with the draft WP (+ any attachments, procedures list) and the WP alignment form. The laboratory animal facility will do its utmost to respond within 5 working days after receipt of the WP by the IvD/AWB.

Contact person Animal facility

- 7) If desired, the animal facility's contact person may directly contact the SD to ask for the facility-specific forms (for example, when registering a project).
- 8) An internal evaluation round of the WP takes place through the animal facility's contact person. The comments on the WP are collected and put on the WP alignment form by the animal facility's contact person. The contact person sends the WP + alignment form to the IvD/AWB.

⁴ Contact persons Animal facilities:

- GDL: Compliance (compliance@uu.nl)
- DGL: Hanke Bons (t.g.denouden@uu.nl)
- DGP: Robin van den boom (r.vandenboom@uu.nl)
- DGG: Marianna Tryfonidou (m.a.tryfonidou@uu.nl)
- Biology/Kruyt; fish: Rudiger Schulz (r.w.schulz@uu.nl);
- RMI (Stratenum): Mieneke Luijendijk (m.c.m.luijendijk@umcutrecht.nl)

IvD

- 9) The IvD/AWB dossier holder edits the comments received into a clear set of comments, understandable for the SD, and sends it by e-mail to the SD.
- 10) If desired, the IvD/AWB dossier holder can organise an in-person meeting between the IvD/AWB, one or more evaluators and the SD to explain the comments and/or the WP to discuss any problems or unclear elements.

Study Director (SD)

- 11) Using the comments from the IvD Utrecht, the SD will modify the WP, provides a response on the alignment form and returns the WP and the alignment form to the IvD Utrecht.

IvD

- 12) If needed, a feedback round (to evaluators and SD) can follow, if the modified version does not correspond to the modifications intended by the IvD Utrecht.
- 13) Once the animal facility's contact person (and others involved with the internal evaluation) approve the WP ('Seen by animal facility' + date), it is sent to the IvD Utrecht.
- 14) After the IvD Utrecht has approved the WP, it sends the work protocol (PDF) (+ 'approved IvD Utrecht' + 'approved by Principal Investigator' (PI) + stamp with IvD Utrecht logo) to the SD with a cc: to the animal facility's contact person and the PI.

SD and Animal facility Contact person

- 15) In consultation with the SD and the Contact person Animal facility, the animals can now be ordered, and the research can start.

IV. Work Protocol - Cover page

Stipulations CCD en IvD

Stipulations that the CCD has set in the project decision and stipulations of the IvD for this specific Work Protocol.

Does this concern an animal experiment?

This work protocol form must also be used if there is not an animal experiment. For example, health monitoring or procedures using animals as part of education labelled 'under-the-threshold' are still evaluated by the IvD/AWB and the DEC. In these cases, tick 'no'.

Type of experiment (according to CCD application)

Choose from the menu, e.g. no. 1 or n/a.

Title of the work protocol

Include in any case keywords in the title of the work protocol corresponding to the title of the type of experiment as stated in the project licence.

Project number (CCD)

The number on the project licence, issued by the CCD.

Principal Investigator(PI) for project

The principal investigator (PI) is the person named on the project licence application form. This person has final responsibility for the entire project and thus for the animal experiments as well.

Project title

Title of the project as stated on the CCD project licence.

End date CCD licence

End date as stated on the CCD project licence.

Work protocol number

This number is assigned by GDL, RMI or the IvD.

Optional: experiment code location

Laboratory animal facilities have the option to add their own experiment code to the work protocol.

Animal facility

Choose a facility from the pull-down menu.

GDL section; 1st and 2nd choice

When an experiment is performed in the GDL, it can happen that it is performed at 2 different GDL sections. If applicable, indicate by choosing one facility from the pull-down menu at each of 'option 1' and 'option 2'. If the experiment is only being conducted at 1 facility or section, then fill in only 'option 1'.

Planned start date: planned end date:

Indicate the planned start and end dates of the work protocol.

Will animals be used in experiments and/or registered as laboratory animals before weaning?

Indicate here if animals will be handled before weaning and thus must be registered as laboratory animals.

Are biological agents (GMO/non-GMO) and/or biological products and/or biological test substances and/or ionising radiation being used?

If the experiment involves working with biological agents, biological products, test substances, cytostatics, substances labelled radioactive, ionising radiation, non-standard feed, etc., then fill out the 'Overview of Hazardous Substances' and add an RIE (Risk Inventory and Evaluation) for the substance(s) concerned to indicate the risks to employees and others (also the environment and other studies), and what additional measures must be taken. This must be evaluated by the appropriate Biological Security Officer or BSO (Biologische Veiligheidsfunctionaris (BVF) in Dutch) or Radiation expert (Stralingsdeskundige). All forms for RIEs can be found at www.uu.nl/gdl (direct to [Forms/procedures \(in Dutch\)](#)). Also see chapter VI: Guide - Hazardous Substances.

Study Director (SD) and Alternate Study Director (ASD)

The Study Director (SD) is the person responsible for the design and execution of the animal experiment as described in the work protocol. This can be the PI named in the application form for the project licence or another researcher involved with the project (such as a PhD student). Both the PI and SD must be authorised in accordance with article 9 of the Experiments on Animals Act. The Alternate Study Director (ASD) is the person who can replace the SD in handling and doing procedure(s) on the animals and who can act as a contact person if the SD is not reachable in circumstances which necessitate consultation (such as welfare problems/illness among the animals). Both a SD and ASD must always be listed on the work protocol (+ mobile telephone number). The ASD and others may be authorised in accordance with either article 9 or article 13.f.2. The people named as SD, ASD or co-workers must all be registered with the IvD Utrecht. Those who do not have the proper authorisation must apply to the IvD/AWB for an exemption.

Departmental laboratory animal coordinator

The department's laboratory animal coordinator may wish to see the work protocol in order to get a clear idea of how many animals will be used per project, which studies are ongoing, what projects are being concluded, or to check invoices, etc.

Contact person animal facility

This person is designated by the laboratory animal facility. At times when the animal facility's contact person is unavailable or unreachable, he or she is replaced by the unit head or another person from the animal facility.

Contact person animal section

To facilitate practical matters (contacts on the work floor, work arrangements, hotline for reporting sick/dead animals, etc.) the laboratory animal facility lists the name of the animal section's contact person.

After conclusion of the work protocol, please send original study dossier and raw data to the address below:

Supply the postal address (of SD) to which the work protocol must be sent after conclusion. Send a welfare evaluation within 2 weeks of conclusion of the WP to info@ivd-utrecht.nl (with cc: to the laboratory animal coordinator of your department). See Concluding the work protocol (top of title page: please tick box, initial and date).

Seen and approved by Principal Investigator responsible for the project

The PI for the project fills in his/her name and date to indicate approval.

Seen and approved by the animal facility

The IvD/AWB submits the WP to the contact person of the facility where the experiment is being conducted (see Routing WP). The animal facility can then evaluate whether the experiment can be conducted at this facility (housing, assistance, safety aspects). After the WP has been seen and approved by the animal facility, the animal facility's contact person ticks 'Seen by animal facility'. If applicable the BSO can discuss RIEs separately from the routing WP with the RE. Indicate on the alignment form that the RIEs must still be discussed.

Approved IvD Utrecht

Once the WP has gone through the routing process and has been approved by the animal facility and the IvD Utrecht, the IvD Utrecht ticks 'Approved IvD Utrecht', with the date, adds a stamp and then sends the WP (as a PDF) to the SD and cc: to the PI and the animal facility's contact person. The WP can now be carried out.

V. Explanatory notes of the questions

1. Description of the experiment and statistics

(see [Guide to experimental design and statistics](#)):

1.a. Abstract. Also give a short summary in simple Dutch.

1.b. What is the specific research question in this work protocol and how does this fit in the research strategy?

1.c. Experimental design and statistics

1.d. Is this a randomised experiment?

1.e. Is this a blinded experiment with respect to animal treatment and analysis of the results experimental groups?

2. Animals

a. Species to be used – Choose

Only one species can be chosen for each work protocol: if you choose 'Other' from the menu, please give a reason. A, B, and C can be used to indicate different genotypes.

Breed/strain name

Use the official strain name as stated on the supplier's website or in catalogue, if possible adding the supplier's stock/reference number(s).

GA

Indicate here if the animals of the species to be used are genetically modified. The SD is personally responsible for including these animals in a notification/licence and risk assessment report.

Immunocompetent

Indicate here if the animals to be used are immunocompetent (yes/no). If it is not known, for example if it is not known beforehand if the offspring of a new cross will be immunocompetent, fill in 'unknown'.

Age/weight

Fill in the desired age and/or weight.

***If genetically modified, please provide corresponding IG number:**

For working with GMOs, an IG (restricted use or Ingeperkt Gebruik) notification/licence must be applied for from Ministry of Infrastructure and Water Management via the GMO Office. The IG number is on your notification/licence.

Category for IG licence issued after 1 March 2015:

The category is assigned according to the assessed risk of the GMO used.

b. Justify the choice of species, strain(s), sex and, if applicable, the reason for the use of the use of transgenic/k.o. animals

Keep in mind the reasoning in the project proposal in your explanation. The explanation in the WP must not in any way contradict the explanation in the project proposal.

c. Re-use

Indicate whether animals that have been used previously in another work protocol (experiment or practicum) may be used. 'Re-use' of an animal is possible when another animal can be used just as well for a follow-up experiment as that animal. If information is available about an animal that is important for the next experiment, it may not be re-used. Instrumentation of an animal (e.g. telemetry) or a previous operation (e.g. gonadectomy) can make this animal especially (i.e. more so than another animal) suitable for another experiment. This is not called re-use in these situations either.

WP number – level of discomfort

In cases of re-use, indicate the WP number under which the animals were previously used, and with what level of discomfort. The IvD Utrecht will assess whether re-use is allowed (see Wod Article 1e).

d. Surplus animals

Indicate here whether or not you can use surplus animals, and support your choice. The establishment licence holders UU and UMC Utrecht are trying to cut the numbers of surplus animals (primarily breeding surpluses) by encouraging such measures as the use of both sexes and more efficient breeding policy.

3. Housing

Group housing

Choose yes (more than 1 animal) or no (individual housing). Since individual housing is considered a discomfort, explain (below in the table) why animals must be housed individually.

Bedding

Choose the animals' bedding from the menu, including 'standard laboratory animal facility', 'no bedding' or 'other (please clarify)'. If you choose 'no bedding' (considered a discomfort), explain (below in the table) why. *If 'other (please clarify)', specify/concretise desires/requirements for housing in explanation.*

Enrichment

Choose one of the possible choices on the menu. *If 'none' or 'other (please specify)', specify and explain (below in table).*

Food

Choose one: 'standard laboratory animal facility', 'GDL treated' (GDL food, irradiated or autoclaved) or 'other (please clarify)' (e.g. food from a different supplier). If a ration is given, choose 'other (please clarify)' and specify and explain in the explanation field (below in table). Treated feed (irradiated or autoclaved) can be used, if available, in breeding.

In case of non-standard food/water: please fill out the 'Hazardous Substances Statement'.

If food with study-specific additives, or food from a different supplier, is used in the laboratory animal facility, or if additives are put into the drinking water, fill in the 'Hazardous substances overview'. For the GDL/RMI: it will be judged by the biological security officer of the GDL, along with the applicable RIE for biological agents. The GDL also requires food from a different supplier to be treated (irradiated 20 kGy or autoclaved). If food cannot be treated, an experiment of this type will only be allowed to take place in the infection unit (behind a negative pressure barrier), due to the possible risk of presence of undesirable micro-organisms.

Water

Choose: 'standard laboratory animal facility' or 'other (please clarify)': e.g. autoclaved tap water, which is often used with immunodeficient animals. If 'other (please clarify)', specify and explain in the explanation field (below in table).

Housing type

Available housing types: open (cage), filter top cage, IVC, Isocage, Isolator, box, paddock, open yard, etc. *If 'other (please clarify)', specify and explain in the explanation field (below in table).*

Restriction level

Choose: none, D-I, DM-I, DM-II of DM-III. The restriction measures of an animal housing unit with restricted use level D-I are aimed at preventing escape of genetically modified animals kept in it. The animal housing units with restricted use levels DM-I through DM-III are suitable for activities with animals (whether or not genetically modified) in association with genetically modified micro-

organisms. Where applicable, restriction levels are also stated on the IG notification/licence. If use is made of several combinations, i.e. of animals (whether or not genetically modified) in combination with micro-organisms of different category levels, state here the highest restriction level.

4. Antibiotics, anaesthesia and analgesics

a. Will antibiotics and/or other anti-microbial prophylactics and/or therapeutics be administered?

If you have questions or need advice about antibiotics or other anti-microbial substances (including anti-mycotics, anti-parasitics, etc.) please contact one of the GDL veterinarians, e-mail dierenartsenGDL@uu.nl, or through the IvD Utrecht.

b. Will anaesthesia be administered?

If you answer 'Yes' please fill in the table.

The IvD Utrecht recommends standard dosages for anaesthesia, for example for isoflurane:

- 3-4% for induction in general anaesthesia
- 1-1.5% to maintain sedation
- 1.5-2% to maintain general anaesthesia
- 5% for induction/maintaining terminal anaesthesia

If using non-standard, special anaesthesia, please provide an explanation. If you have questions or need advice about anaesthesia, please e-mail the GDL veterinarians (dierenartsenGDL@uu.nl) or the IvD/AWB (<mailto:info@ivd-utrecht.nl>).

c. Will analgesics be administered?

If you answer 'Yes', then fill in the table. Here as well, the IvD Utrecht recommends using standard dosages if possible, and refers to standard methods (SOPs, SHEQ documents, literature references). If no analgesics will be used, please explain. Although it may be that the procedure(s) is (are) painless, it may also be that pain is indicated but that analgesics are not being administered for other reasons (for example, it interferes with the readout). This must be supported with a literature reference. If you have questions or need advice about analgesics: mail the GDL veterinarians (dierenartsenGDL@uu.nl) or the IvD Utrecht (<mailto:info@ivd-utrecht.nl>).

5. Describe the consecutive procedures

a. Acclimatisation

The IvD Utrecht recommends a standard acclimatisation period of 1 week. Keep in mind the possibility that, depending on the origin and history of the animals and the type of experiment, a non-standard duration of the acclimatisation period will be a point of concern and consideration for the IvD Utrecht.

b. Schedule of experimental procedures

Please use a relative indication of time (e.g. day -3, day 0 (start of actual experiment), day 3, etc.) for the timeline! Please note: the actual listing of experimental procedures (besides welfare observation) is in the procedures list. In contrast to Table 5.b, a strict chronological order (time axis) of procedures to be performed per group must be described in the **procedures list**, to enable inspectors (from the NVWA, IvD Utrecht, etc.) to easily see if planned experimental procedures are being carried out in line with the planning in the WP. Points for experiment-specific welfare observations are indicated in Table 6.b, and the findings are written down in the welfare log. **Estimated level of discomfort:** choose **mild, moderate, severe, terminal**. See examples in annex VIII of EU Directive 2010/63/EU, which can be found at www.ivd-utrecht.nl.

Table 5.b. (Example as illustration of a completed table)

Line	Relative day in experiment	Exp. group	Description of the procedure	Duration of procedure	Description of discomfort during and as a result of the procedure	Duration of discomfort	Estimated discomfort	Who (see pg.1)
1	D3, D6 and D14	1, 2, 3	100 µl blood drawn via vena saphena	5 min	Discomfort from restraint and blood draw	5 min	mild	RE
2	D0, D7	1, 2, 3	Subcutaneous injection of 2 ml PBS (group 1) vaccine X (groups 2 and 3)	5 min	Discomfort from restraint and injection	5 min	mild	RE
3	D15	1, 2	Challenge with AI 6log ₁₀ tcid 50 µl, in 1 ml/nostril, intranasal drops	5 min	Discomfort from restraint and intranasal drops	5 min	mild	RE
4	D15	3	Challenge met AI 4log ₁₀ tcid 50 µl, in 1 ml/ nostril, intranasal drops	5 min	Discomfort from restraint and intranasal drops	5 min	mild	RE
5	D17, D19, D21, D23 and D25	1, 2, 3	Sample collection using throat swab	2 min	Discomfort from restraint and throat swab	2 min	mild	RE
6	D28	1, 2, 3	Euthanasia	1 min	Discomfort from restraint and killing method	1 min	mild	RE

6. Welfare check and humane endpoint

a. Regular welfare check

The assumption is that at all times (from the time that the animal enters the animal facility/housing unit) the animal and the housing unit are checked **daily**. This is a routine check for calamities. Every day, the caretaker checks the laboratory animals at the housing unit (cage) level. The daily check is nothing more than a quick, rough check – the cages are scanned from a short distance so as not to disturb the animals. This daily check is noted in a log or room log. **At least 1x per 7 days** there must be a thorough welfare check of each animal, based on **behaviour, posture, gait/mobility, nutritional status (food intake, weight loss), care status (grooming, self-mutilation) and other obvious general clinical symptoms**. The findings from this welfare check are noted in the welfare log.

b. Additional welfare check within the scope of possible experiment-specific clinical symptoms

Depending on the experiment, so-called experiment-specific clinical symptoms can occur which necessitate more extensive welfare assessments in addition to the weekly welfare checks described in 6.a. If 'yes', then please indicate in the table below:

- At what points which laboratory animals must be assessed. Describe the experiment-specific clinical symptoms and any resulting general symptoms⁵ expected at this/these point(s) and/or if this involves an additional extensive regular welfare assessment. If possible, quantify a clinical symptom/parameter, e.g. cubic volume of a tumour, circumference of a swollen knee joint.
- Also indicate the expected degree of discomfort (mild, moderate, severe).

Table 6.b. Points (relative day of experiment) of observation and the experiment-specific clinical symptoms that need to be assessed.

(Sample table, as illustration)

Line	Day/period of experiment	Observation frequency	Experimental group	Experiment-specific clinical symptoms to be assessed	Estimated discomfort	Who (see pg.1)
1	D19	2x	1	Minor nasal discharge/redness in nasal mucosa, mild cough, no fever	mild	RE
2	D21	2x	1	Moderate nasal discharge/redness in nasal mucosa, moderate cough, fever (under 40°C)	moderate	RE
3	D21	1x	2 and 3	Minor nasal discharge/redness in nasal mucosa, mild cough, no fever	mild	RE

⁵ Experiment-specific clinical symptoms (e.g. size of tumour, swelling in knee joint) can influence the general parameters (e.g. resulting in deviant behaviour, posture, gait/mobility, nutritional status (food intake, weight loss), and/or care status (grooming, self-mutilation). If applicable and relevant, you can list such general symptoms. Consult the IvD if necessary.

Line	Day/period of experiment	Observation frequency	Experimental group	Experiment-specific clinical symptoms to be assessed	Estimated discomfort	Who (see pg.1)
4	D23	3x	1	Heavy nasal discharge /redness and scabs forming in nasal mucosa, frequent coughing, fever (40-41°C)	severe	RE
5	D23	3x	2	Moderate nasal discharge/redness in nasal mucosa, moderate cough, fever (under 40°C)	moderate	RE
6	D23	1x	3	Minor nasal discharge /redness in nasal mucosa, mild cough, no fever	mild	RE
7	D25 and D27	3x	1	Heavy nasal discharge /redness and scabs forming in nasal mucosa, frequent coughing, fever (40-42°C)	severe	RE
8	D25 and D27	3x	2	Heavy nasal discharge /redness and mild scabs in nasal mucosa, moderate cough, fever (under 40°C)	moderate	RE

c. Humane end point / Premature withdrawal of animal(s)

The primary goal of a humane end point is to prevent avoidable and unnecessary discomfort in the animal. The humane end point is the point at which it is necessary to exclude the animal from the experiment, reduce its pain and/or discomfort, or euthanize the animal. This point has been reached if:

I. The discomfort of an individual animal that is inherent to the experiment exceeds the maximum discomfort described in the project.

Indicate as precisely as possible on the basis of which clinical symptoms and/or other parameters/criteria (e.g. weight, metastases (bioluminescence)) will be used to determine that the humane end point has been reached. Quantify if possible a clinical symptom/parameter, e.g. cross-section of a tumour, the circumference of a swollen knee joint, the percentage of weight loss per day, etc.

Describe the HEP also in Dutch.

II. The scientific goal has been reached.

Describe at the level of the individual animal when the scientific end point has been reached and the criteria are for excluding an animal from the experiment. For example: a set tumour size has been reached, or metastasis (primary readout) has been identified through imaging.

III. The scientific goal can no longer be reached

Nothing needs to be noted here. It is however an **important point for attention**. For example, no significant results can be obtained due to dropouts, or the readout has been influenced by external factors (e.g. infections as a result of bite wounds when mice fight with each other), and as a result the animal can no longer provide reliable results. If this occurs, make a note in the welfare log and report it to the IvD Utrecht!

7. Estimated maximum discomfort by experimental group

Indicate here what the estimated maximum discomfort is for each experimental group. Please use a separate line for each experimental group (see example below).

Experimental group	Estimated maximum discomfort	Substantiate discomfort level (on the basis of type and number of procedures – see Table 5.b., and clinical symptoms – see Table 6.b.)
1	Severe	(see total of procedures in Table 5.b. combined with clinical symptoms resulting from AI viral challenge control)
2	Moderate	(see total of procedures in Table 5.b. combined with clinical symptoms resulting from AI viral challenge; high challenge dosage)
3	Moderate	(see total of procedures in Table 5.b. combined with clinical symptoms resulting from AI viral challenge; low challenge dosage)

8. Ill and dead animals during the experiment

Unexpected ill/dead animals must always be reported to a animal facility employee and the IvD Utrecht.

Investigating the cause of illness/death is strongly advised. The results of this investigation may help to prevent illness/death in future research, and it can provide information that is relevant to the health status of other animals at the animal facility.

9. Destiny of animals at the end of the experiment

Destination

Choose: euthanasia or re-use. If they will be available as surplus laboratory animals which can be re-used, you must state the date at which they will be available and other information in the 'clarification when re-use is possible' field. In a very few cases, animals may qualify for adoption/re-homing. See [Adoption](#). The researcher reports the animals as surplus laboratory animals to the IvD Utrecht (info@ivd-utrecht.nl) 2 weeks before the date they will be available. See also the [policy paper on surplus animals](#).

Method of euthanasia

Choose one, e.g. mouse/rat/bird cervical dislocation. If you do not see the method being used, then choose 'other, see explanation' and explain the method in the explanation field. Do make sure that the method indicated is in line with a) the information given in the project proposal and b) the methods listed in [Annex IV of EU Directive 2010/63/EU](#).

VI. Guide – Hazardous Substances Overview

Are biological agents (GMO yes/no) and/or biological substances and/or test substances and/or non-standard feed and/or ionizing radiation be used? All forms needed for Risk Inventory and Evaluation (RIE's) can be found on www.uu.nl/gdl. All RIE's are assessed for safety for humans (employees/researchers), other studies and the environment.

Agent number PRIS: If the hazardous substance has previously been assessed, use this number. This will speed up the evaluation procedure; however, the RIE still must be attached.

- A. Biological agents, both GM and non-GM (micro-organisms, transduced cells/cell lines). For GM biological agents the researcher him/herself is responsible for applying for an IG notification/licence and keeping track of the risk assessment report. The same safety levels and required procedures are used for the wild types.
- B. Biological products of human or animal origin (e.g. organs, tissues, cells, cytokines, antibodies) must be reported so that they can be assessed for any risk potential for the microbiological status of the laboratory animal facility.
- C. Test/auxiliary substances (toxic substances, cytostatics, pharmaceuticals, etc.). Specific information about the test substances is important for evaluating if additional measures need to be taken in order to work safely. If an MSDS (Material Safety Data Sheet (safety info from the supplier), or an information leaflet for pharmaceuticals is available, it can be referred to in the RIE. Attach the MSDS/leaflet.
- D. Non-standard feed. The GDL has regular feed suppliers, who are allowed to supply feed. If feed is involved from another supplier or special additives are added to feed, these must be assessed to see if they constitute a risk to the microbiological status of the GDL and/or employee safety.
- E. Describe here any possible **side effects including discomfort** to animals of the substances listed in A through D.
- F. Ionising radiation. The use of ionising radiation falls under the supervision of the radiation experts of GDL, DGK and UU.

A written internal permission (Schriftelijke Interne toestemming or SIT) form is needed for both or open and closed sources. For use of open sources, a new SIT must usually be requested from the coordinating radiation expert of the UU, for the use of existing closed sources, refer to the applicable SITs:

- GDL C-arm: 2578 09.065 GDL SIT BV Pulsera release 2-2 NZS92 instrument
- GDL infection unit X-ray cabinet: 1797 DGK GDL SIT Therapie Smart 225 instrument
- GDL micro CT scanner: 2903 DGK GDL SIT micro CT scanner instrument
- GDL Fixed C- arm OK 6: 2919 DGK GDL SIT Allura Aper FD20 NZA 135 instrument

For an overview of all SITs, see <https://uniteams.services.uu.nl/ubd/SBE/default.aspx>

VII. Guide to registration with NVWA

Please fill in provisionally, using the code numbers according to the NVWA registration for carrying out the work protocol in the table. A guide to the codes is on pg. 10. After completion of the work protocol you must transfer the codes from this table, after modifying them to reflect the actual situation as it occurred (more/less discomfort than estimated, animals who died spontaneously or were euthanized because a humane end point was reached) in the welfare evaluation form. The welfare evaluation form must be sent **within two weeks of completion of the work protocol**, including the table with in column 12 the **actual discomfort observed** to the IvD Utrecht and the laboratory animal coordinator of your own department.