

## Animal Use Alternatives Literature Search Example #1

Created by NAL's Animal Welfare Information Center (AWIC)  
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### Example 1

#### [Step 1: Identify Areas to Potentially Implement Alternatives](#)

When planning an alternatives literature search, review the protocol (a proposed research study) and identify areas of the protocol that could incorporate the 3Rs (replacement, reduction, and refinement of animal use). Has the Principal Investigator (PI) considered methods/techniques that could reduce the number of animals used, minimize pain and distress, or in some cases, eliminate the use of animals altogether? These questions will provide the groundwork for your search strategy.

Below is a sample protocol for a study that uses ferrets to develop and evaluate an H5N1 avian influenza vaccine.

*NOTE: The highlighted text and numbers in parenthetical statements at the end of each section, correspond with the list of the 3Rs examples directly below the protocol.*

#### **Development of a ferret H5N1 influenza system for testing new vaccines**

As part of the preparation for a potential influenza pandemic, development of a vaccine to protect human health has become a priority. Ferrets are the preferred animal model for influenza research due to human similarities in lung physiology, clinical signs and other biological factors. Therefore, the aim of the study is to evaluate the efficacy of a new influenza vaccine for humans using ferrets.

#### **Materials and Methods**

1. Animals: 40 male or female ferrets, age 8-10 months **(1 and 2)**
2. Virus suspension

#### **Methods**

- Ferrets are housed individually **(3)** in bioclean portable laminar flow clean room enclosures.
- Challenge: Ferrets will be anesthetized with ketamine (25 mg/kg), xylazine (2 mg/kg), and atropine (0.05 mg/kg) by the intramuscular route **(4)**, then held vertically and 0.5 to 1 ml of the virus suspension will be pipetted into their nostrils. **(5)** Control animals will receive an equivalent dilution (1:30) of noninfectious allantoic fluid.

- Nasal flushes: On days 1, 3, 5, and sometimes 7, post-challenge, ferrets will be lightly sedated and restrained. (6) A small amount of fluid will be dribbled into their nostrils to elicit sneezing. The nasal secretions will be collected in a dish.
- 1 mL of blood is collected from the anterior vena cava of sedated ferrets on the same days as the nasal flushes.(7)
- Monitoring health: Ferrets will be weighed daily after challenge and examined for disease twice each day. (8) Animals are euthanized when they show severe signs of disease or at the end of the study (14 days post-challenge). Two animals will be euthanized on days 1, 3, 5, and 7 days post-challenge to internally evaluate the progression of the disease.(9) Animals will be euthanized by anesthetizing them with ketamine-xylazine followed by intracardiac pentobarbital. (10)

Each of the following questions address at least one of the 3Rs:

- (1) **Replacement—Models:** Can this study be conducted in a non-animal model?
- (2) **Reduction—Study Design:** Does this protocol duplicate published research? Is the number of animals proposed reasonable for this type of study? Can the researcher reduce the number of animals used, but still obtain statistical significance?
- (3) **Refinement—Social Housing:** Ferrets are a social species and can become stressed when housed individually. Can ferrets be socially-housed without affecting the study? Furthermore, what (if any) environmental enrichments could be included?
- (4) **Refinement—Pain and Distress:** Review the literature and [Plumb's Veterinary Drug Handbook](#). Are the proposed anesthetic/analgesic drugs and dosages adequate for ferrets?
- (5) **Refinement—Challenge Method:** Are there other ways of challenging ferrets that are less-invasive? What are the advantages and disadvantages to these methods?
- (6) **Refinement—Handling/Training:** Could the ferrets be trained for handling during the nasal flushes rather than lightly sedated and restrained?
- (7) **Refinement—Blood Collection Method:** Is the vena cava vein the best and most humane blood collection site for ferrets?
- (8) **Refinement—Pain and Distress:** What are the behavioral signs of pain and distress in ferrets? Are there other ways to monitor health and pain/distress?
- (9) **Reduction/Refinement—Imaging:** Could disease progression be observed in the same animal through imaging or other methods instead of euthanizing animals at specified time points?
- (10) **Refinement—Euthanasia/Humane Endpoints:** Review the literature and current [AVMA \(American Veterinary Medical Association\) Euthanasia Guidelines](#). Is this the most humane method of euthanasia for ferrets?

## [Step 2: Finding Databases and Grey Literature](#)

One database does not cover 100% of the literature on a particular subject area or all types of materials (journals, books, meetings, conferences, etc.). Therefore, it is important to search multiples databases to capture all/most of the information needed. When choosing which databases to search, think about your academic discipline, the subject matter of your protocol, and what types of materials you want to find. You can also use other resources such as grey literature (e.g. conference abstracts, theses/dissertations, reports, newsletters, etc.), books, [National Agricultural Library](#) (NAL) and [Animal Welfare Information Center](#) (AWIC) webpages, other 3Rs websites, or Google Scholar.

For this search, we will use the following bibliographic databases: PubMed, Web of Science, and EBSCO.

## [Step 3-4: Developing a Search String](#)

We will use **Question 5** from the protocol to build our search string:

**Refinement—Challenge Method:** Are there other ways of challenging ferrets that are less-invasive? What are the advantages and disadvantages to these methods?

Use your question to come up with the different keywords/concepts that express the main topics and ideas you are searching for (see 1 – 4 below). Then brainstorm keywords and synonymous terms for that concept:

1. Animal or species keywords (*ferret*, *Mustela putorius furo*, *Mustela putorius*)
2. Action or verb keywords (challenge, infect, administer, inoculate, expose)
3. Disease keywords (H5N1 influenza, avian influenza, avian flu, bird flu, avian flu virus, bird flu virus, H5N1 virus)
4. 3Rs keywords (less invasive, non-invasive, minimally invasive, stress-free, welfare, well-being, refinement, non-aversive, painless, less aversive, low stress, humane, method, procedure)

\*Note: When including 3Rs keywords, do not limit yourself to words such as, "alternatives" or the 3Rs (refinement, reduction, replacement). Include additional terms that are examples of these concepts. In this example, we've included keywords such "less invasive", "non-invasive", and "non-aversive" since we're interested in challenge methods that are potentially less stressful.

Then, construct your search string by using syntax tools (Boolean operators, parentheses, truncation, etc.) to link your keywords, their synonyms, and concepts together.

## Building a Search Using the PubMed Advanced Search Builder:

1. Go to [PubMed](#).
2. Select **Advanced** below the search box. This will take you to PubMed's Advanced Search Builder.
3. Below **Add terms to the query box**, select **Title/Abstract** from the drop-down menu.
4. Add your animal/species concept keywords to the search box:
  - a. In the **Enter a search term** box add, **ferret\* OR "Mustela putorius furo" OR "Mustela putorius"**. Select the blue **ADD** button. The keywords should populate in the "Query box".
5. Next, add your action/verb concept keywords to the search box:
  - a. In the **Enter a search term** box add, **challenge OR infect\* OR administ\* OR inoculat\* OR expos\*** and select the blue **AND** button. The keywords should populate in the "Query box".
6. Then, add your disease concept keywords to the search box:
  - a. In the **Enter a search term** box add, **"H5N1 influenza" OR "avian influenza" OR "avian flu" OR "bird flu" OR "avian flu virus\*" OR "bird flu virus\*" OR "H5N1 virus"** and select the blue **AND** button. The keywords should populate in the "Query box".
7. Finally, add your 3Rs concept keywords to the search box:
  - a. In the **Enter a search term** box add, **"less invasive" OR noninvasive OR "minimally invasive" OR "stress-free" OR welfare OR "well-being" OR refinement OR 3Rs OR "three rs" OR "non-aversive" OR painless OR "less aversive" OR "low stress" OR humane OR method\* OR procedure\*** and select the blue **AND** button. The keywords should populate in the "Query box".
8. Now that all four of your concept groups are added into the query box, select the blue **Search** button to execute the search. View the [search results](#).

Here is the full search string:

```
((ferret*[Title/Abstract] OR "Mustela putorius furo"[Title/Abstract] OR "Mustela putorius"[Title/Abstract]) AND (challenge[Title/Abstract] OR infect*[Title/Abstract] OR administ*[Title/Abstract] OR inoculat*[Title/Abstract] OR expos*[Title/Abstract])) AND ("H5N1 influenza"[Title/Abstract] OR "avian influenza"[Title/Abstract] OR "avian flu"[Title/Abstract] OR "bird flu"[Title/Abstract] OR "avian flu virus*" [Title/Abstract] OR "bird flu virus*" [Title/Abstract] OR "H5N1 virus"[Title/Abstract])) AND ("less invasive"[Title/Abstract] OR noninvasive[Title/Abstract] OR "minimally invasive"[Title/Abstract] OR "stress-free"[Title/Abstract] OR welfare[Title/Abstract] OR "well-being"[Title/Abstract] OR refinement[Title/Abstract] OR 3Rs[Title/Abstract] OR "three rs"[Title/Abstract] OR "non-aversive"[Title/Abstract] OR painless[Title/Abstract] OR "less aversive"[Title/Abstract] OR "low stress"[Title/Abstract] OR humane[Title/Abstract] OR method*[Title/Abstract] OR procedure*[Title/Abstract])
```

## [Step 5: Modifying a Search String](#)

Searching is a trial-and-error process. If you are finding a lot of irrelevant results, you will need to modify your search string. There are several options for modifying a search string, so choose one or more modifications based on the results. Also, there is no "right way" to modify a search string, so how you choose to modify won't necessarily be the same every time.

For this example search, we browsed through the titles and abstracts looking for relevant citations. There are some relevant citations in the first set of results. They use keywords such as natural, aerosol, and airborne. One way to modify a search string is to add new keywords/synonyms that are found while reviewing the results, so these terms can be added to the 3Rs search string.

Also, the relevant citations have ferret(s) in the title, while many irrelevant citations don't have ferret(s) in the title. Another way to modify a search string is to change the field being searched. In this example, we will search the ferret search string in the Title field rather than the Title/Abstract field.

After making these two modifications to the search string, we get the following search string and **results**:

```
((ferret*[Title] OR "Mustela putorius furo"[Title] OR "Mustela putorius"[Title]) AND (challenge[Title/Abstract] OR infect*[Title/Abstract] OR administ*[Title/Abstract] OR inoculat*[Title/Abstract] OR expos*[Title/Abstract])) AND ("H5N1 influenza"[Title/Abstract] OR "avian influenza"[Title/Abstract] OR "avian flu"[Title/Abstract] OR "bird flu"[Title/Abstract] OR "avian flu virus*" [Title/Abstract] OR "bird flu virus*" [Title/Abstract] OR "H5N1 virus"[Title/Abstract])) AND ("less invasive"[Title/Abstract] OR noninvasive[Title/Abstract] OR "minimally invasive"[Title/Abstract] OR "stress-free"[Title/Abstract] OR welfare[Title/Abstract] OR "well-being"[Title/Abstract] OR refinement[Title/Abstract] OR 3Rs[Title/Abstract] OR "three rs"[Title/Abstract] OR "non-aversive"[Title/Abstract] OR painless[Title/Abstract] OR "less aversive"[Title/Abstract] OR "low stress"[Title/Abstract] OR humane[Title/Abstract] OR method*[Title/Abstract] OR procedure*[Title/Abstract] OR natural[Title/Abstract] OR aerosol*[Title/Abstract] OR airborne[Title/Abstract])
```

After reviewing the new list of citations, we have minimized the irrelevant results and have several relevant citations, so this completes our search in PubMed. Notice how we had to make multiple modifications to our search to get our final search string that had the most relevant results. The 4 articles below are the most relevant by using aerosol inoculation and other natural exposure routes instead of sedating the ferrets for inoculation:

Belser, J. A., Gustin, K. M., Katz, J. M., Maines, T. R., & Tumpey, T. M. (2014). Influenza virus infectivity and virulence following ocular-only aerosol inoculation of ferrets. *Journal of Virology*, 88(17), 9647–9654. <https://pubmed.ncbi.nlm.nih.gov/24920819/>

Edenborough, K. M., Lowther, S., Laurie, K., Yamada, M., Long, F., Bingham, J., Payne, J., Harper, J., Haining, J., Arkinstall, R., Gilbertson, B., Middleton, D., & Brown, L. E. (2016). Predicting Disease Severity and Viral Spread of H5N1 Influenza Virus in Ferrets in the Context of Natural Exposure Routes. *Journal of Virology*, 90(4), 1888–1897. <https://doi.org/10.1128/JVI.01878-15>

Gustin, K. M., Belser, J. A., Wadford, D. A., Pearce, M. B., Katz, J. M., Tumpey, T. M., & Maines, T. R. (2011). Influenza virus aerosol exposure and analytical system for ferrets. *Proceedings of the National Academy of Sciences of the United States of America*, 108(20), 8432–8437. <https://doi.org/10.1073/pnas.1100768108>

Tuttle, R. S., Sosna, W. A., Daniels, D. E., Hamilton, S. B., & Lednicky, J. A. (2010). Design, assembly, and validation of a nose-only inhalation exposure system for studies of aerosolized viable influenza H5N1 virus in ferrets. *Virology Journal*, 7, 135. <https://doi.org/10.1186/1743-422X-7-135>

## [Step 6: Records and Citation Management](#)

Keep a record of your keywords, search strings, databases searched, and publication years searched. This allows you to simply update your search when needed in the future, rather than regenerating a list of keywords and building new search strings every time.

You can also use citation management software (CMS) such as EndNote or Zotero to store, organize and utilize the relevant citations you find. CMS helps with generating reference lists, bibliographies, and footnotes. Contact your institution’s librarian to find out which citation managers are available to you. However, if you are a United States Department of Agriculture (USDA) employee and need assistance with using CMS, [contact the National Agricultural Library](#).

## **Example 2**

### [Step 1: Identify Areas to Potentially Implement Alternatives](#)

Step 1 has already been completed above during example 1.

## Step 2: Finding Databases and Grey Literature

For this search, we will use the same bibliographic databases: PubMed, Web of Science, and EBSCO.

## Step 3-4: Developing a Search String

We will use **Question 9** from the protocol to build our search string:

**Reduction/Refinement—Imaging:** Could disease progression be observed in the same animal through imaging instead of euthanizing animals at specified time points?

Divide this question into different concepts (i.e. animal, disease, and 3Rs keywords or search terms):

1. Animal or species keywords (*ferret*, *Mustela putorius furo*, *Mustela putorius*)
2. Disease keywords (H5N1 influenza, avian influenza, avian flu, bird flu, avian flu virus, bird flu virus, H5N1 virus, influenza virus)
3. 3Rs keywords (imaging, scans, magnetic resonance imaging, MRI, computed tomography, CT, positron emission tomography, PET, radiographs, radiolog, telemetry, visualize, biomarkers)

Then, construct your search string by using syntax tools (Boolean operators, parenthesis, truncation, etc.) to link your keywords, their synonyms, and concepts together.

Building a Search Using EBSCO:

Go to the main searching page for EBSCO. Depending on your institution, there may be different ways of accessing EBSCO. If you need assistance, contact your institution's library.

1. Select **Choose Databases** and select the databases you want to search. You must select which databases you want to use before beginning your search.
  - a. For this search, we'll select Agricola, CAB Abstracts, eBook Collection, Zoological Record, Biological Abstracts, Global Health, and Medline. These are databases available to United State Department of Agriculture (USDA) employees through the National Agricultural Library.
  - b. Each library tailors their EBSCO subscription to include databases pertinent to its institution/organization. Contact your librarian to find out what databases you can access through EBSCO.
2. Below the search bar, select **Advanced Search**.
3. In the first search box add, **ferret\* OR "Mustela putorius furo" OR "Mustela putorius"**. In the "Select a Field (optional)" menu to the right, select **AB Abstract**.

4. Select **AND** from the drop-down menu to the left of the second search box.
5. In the second search box add, "**H5N1 influenza" OR "avian influenza" OR "avian flu" OR "bird flu" OR "avian flu virus\*" OR "bird flu virus\*" OR "H5N1 virus" OR "influenza virus"**. In the "Select a Field (optional)" menu to the right, select **AB Abstract**.
6. Select **AND** from the drop-down menu to the left of the third search box.
7. In the third search box add, **imaging OR scan\* OR "magnetic resonance imaging" OR MRI OR "computed tomography" OR CT OR "positron emission tomography" OR PET OR radiograph\* OR radiology OR telemetry OR visual\***. In the "Select a Field (optional)" menu to the right, select **AB Abstract**.
8. Select the green **Search** button.

Here is the full search string:

```
AB ( ferret* OR "Mustela putorius furo" OR "Mustela putorius" ) AND AB ( "H5N1 influenza" OR "avian influenza" OR "avian flu" OR "bird flu" OR "avian flu virus*" OR "bird flu virus*" OR "H5N1 virus" OR "influenza virus" ) AND AB ( imaging OR scan* OR "magnetic resonance imaging" OR MRI OR "computed tomography" OR CT OR "positron emission tomography" OR PET OR radiograph* OR radiology OR telemetry OR visual* OR biomarker* )
```

### [Step 5: Modifying a Search String](#)

After reviewing the titles and abstracts, we notice that "PET" is being used in the context of animals rather than imaging. One way to refine a search is by removing words that produce irrelevant results. In this case, we will remove the word "PET".

After making this modification, we get the following search string:

```
AB ( ferret* OR "Mustela putorius furo" OR "Mustela putorius" ) AND AB ( "H5N1 influenza" OR "avian influenza" OR "avian flu" OR "bird flu" OR "avian flu virus*" OR "bird flu virus*" OR "H5N1 virus" OR "influenza virus" ) AND AB ( imaging OR scan* OR "magnetic resonance imaging" OR MRI OR "computed tomography" OR CT OR "positron emission tomography" OR radiograph* OR radiology OR telemetry OR visual* OR biomarker* )
```

After reviewing the new list of citations, we have minimized the irrelevant results and have several relevant citations, so this completes our search in EBSCO. It is important to note that while we only had to modify the search string once, it may take multiple revisions of your search string to get the most relevant results.

Out of the citations we retrieved, the 5 citations below are relevant because they use imaging rather than euthanizing ferrerts at different time points to study the disease's progression.



Jonsson, C. B., Camp, J. V., Wu, A., Zheng, H., Kraenzle, J. L., Biller, A. E., Vanover, C. D., Chu, Y.-K., Ng, C. K., Proctor, M., Sherwood, L., Steffen, M. C., & Mollura, D. J. (2012). Molecular imaging reveals a progressive pulmonary inflammation in lower airways in ferrets infected with 2009 H1N1 pandemic influenza virus. *PloS One*, 7(7), e40094. MEDLINE. <https://doi.org/10.1371/journal.pone.0040094>

Karlsson, E. A., Meliopoulos, V. A., Savage, C., Livingston, B., Mehle, A., & Schultz-Cherry, S. (2015). Visualizing real-time influenza virus infection, transmission and protection in ferrets. *Nature Communications*, 6(3), 6378–6378. CAB Abstracts. [doi: 10.1038/ncomms7378](https://doi.org/10.1038/ncomms7378)

Karlsson, Erik A, Meliopoulos, V. A., Tran, V., Savage, C., Livingston, B., Schultz-Cherry, S., & Mehle, A. (2018). Measuring Influenza Virus Infection Using Bioluminescent Reporter Viruses for In Vivo Imaging and In Vitro Replication Assays. *Methods in Molecular Biology (Clifton, N.J.)*, 1836, 431–459. MEDLINE. [https://doi.org/10.1007/978-1-4939-8678-1\\_21](https://doi.org/10.1007/978-1-4939-8678-1_21)

Veldhuis Kroeze, E. J. B., Stittelaar, K. J., Teeuwssen, V. J., Dijkshoorn, M. L., van Amerongen, G., de Waal, L., Kuiken, T., Krestin, G. P., Hinkula, J., & Osterhaus, A. D. M. E. (2012). Consecutive CT in vivo lung imaging as quantitative parameter of influenza vaccine efficacy in the ferret model. *Vaccine*, 30(51), 7391–7394. MEDLINE. <https://doi.org/10.1016/j.vaccine.2012.10.008>

Veldhuis Kroeze, E. J. B., van Amerongen, G., Dijkshoorn, M. L., Simon, J. H., de Waal, L., Hartmann, I. J. C., Krestin, G. P., Kuiken, T., Osterhaus, A. D. M. E., & Stittelaar, K. J. (2011). Pulmonary pathology of pandemic influenza A/H1N1 virus (2009)-infected ferrets upon longitudinal evaluation by computed tomography. *The Journal of General Virology*, 92(Pt 8), 1854–1858. <https://doi.org/10.1099/vir.0.032805-0>

### Step 6: Records and Citation Management

Keep records and use citation management software as mentioned above in example 1.