ANIMAL FACILITIES

Providing the highest level of expertise in **Custom Antibodies**

Eurogentec
Experience true partnership

Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter.

American Veterinary Medical Association

https://www.avma.org/kb/resources/reference/animalwelfare/pages/animal-welfare-division-contacts.aspx

Animals have an intrinsic value, let's

Read more about
the directives 2010/63/EU
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

Animal Welfare

have respect for them

For more than 25 years Eurogentec has been a leading supplier of custom antibodies and related services. Although significant advances are constantly made towards the generation of synthetic antibodies and other binding molecules, the immunisation of animal hosts remains the gold standard to produce high affinity antibodies, both monoclonals and polyclonals.

At Eurogentec, animal welfare is our priority and as such, our animal facility -located in south Belgium- fully respects the sternest ethical legislations in force, and even more stringent requirements from our sponsors.

Within the European Union, directive 2010/63/EU, revising the previous Directive 86/609/EEC, was adopted on September 22nd, 2010, and transposed into EU national texts with an effective date starting January 1st, 2013. This new directive calls for the most rigorous and transparent measures in the area of animal experimentation, and is centred on the 3 R's: replacement, reduction and refinement.

Our accreditation is global, and covers our facilities,

our animal care system, and our high staff training. Animal sources are strictly controlled, and statistical data regarding the use of animals are recorded. An internal Ethical Committee (IACUC) is in place, and **the facility is regularly inspected by the belgian national authorities for its compliance regarding animal origin, identification, housing, and welfare.**

Table & lists of the regulations followed by our animal facility regarding animal care, housing and transportation.

The Ethical Committee plays a strategic role. Each new protocol is evaluated at latest one month prior to its implementation. The Ethical Committee retains the right to require protocol adjustment or rejection with the constant goal of improving the animal welfare. Protocols are reviewed every 5 years or earlier in case of problem.

The Ethical Committee is composed by at least seven independant members highly competent in animal health, ethics, alternative methods, statistics, animal welfare, study design and research technics. All members are bound by professional secrecy.

Nowadays we are all conscious that the use of living animals for scientific or educational purposes continues to be necessary to protect human and animal health and the environment but it must only be considered when a non-animal alternative is unavailable.

A particular case applies to the production of monoclonal antibodies, classically performed by the ascites method. It is well established that this method induces stress and pain to the animals and as such, it is now forbidden in several countries including Australia, Germany, Switzerland, the Netherlands and the United Kingdom. Belgium was a pioneer, since the royal decree of April 25, 2004 prohibited the use of ascites method in favour of *in vitro* methods. Accordingly, Eurogentec has acquired a long experience in producing monoclonal antibodies *in vitro*, and our processes are highly optimised.

Since 2000, Eurogentec's Quality Management System has been certified according to ISO 9001.
This certification applies to the following activities: "Development, production and sales of products and services for research and development in Life Sciences".

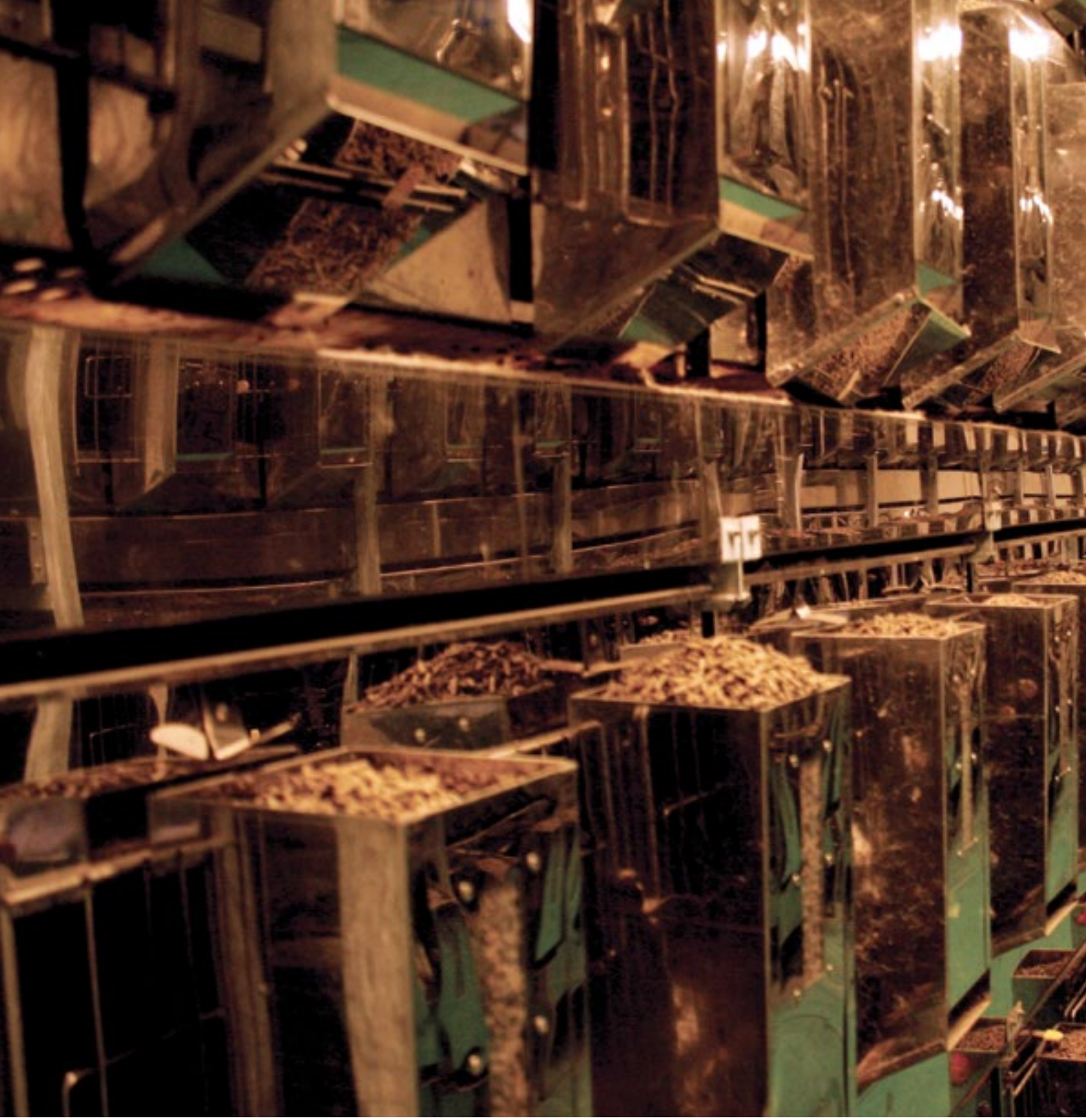
Welfare Legislation for laboratory animals
2010/63/EU
Animal Transportation
01/2005/EU
Pharmacy
RD29/06/199
RD23/05/2000
RD19/12/2002
Others
Agreement STE123
Scientific procedures: Animals Act 1986
2004/21/EU Identification of ovine and caprine species

Table 1: Regulations followed by Eurogentec for animal care, housing and transportation.

The animal facility is recognized by the authorities for using lab animals. It complies with the following association's requirements:

- **Federation of European Laboratory animal Science Associations (FELASA)**
- **UK Home Office Animals Scientific Procedures Act**

We maintain a constant technological watch to comply with the highest standards for animal protection. Based on this watch and fruitful exchanges with experts, a continuous improvement of the animal quality of life in our housings is ensured. Frequent inspections and periodic checks of our animal facility guarantee adherence to these standards.



Each Animal deserves care,

Our facilities can be audited…
Just ask for an appointement:
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Animal Facilities

and protection

Through numerous exchanges with customers and regular audits, Eurogentec's animal facility has become one of the world cleanest and safest animal housings.

II.1 CONTINUOUS IMPROVEMENT

20% Reduction of the number of animals used

- Use of highly adapted and efficient materials;
- Special training for technicians has reduced animal stress and maximized the protocol efficiency;
- Constant improvements of technics and staff management.

Replacement

- Whenever possible, polyclonal antibody production is realised in eggs and monoclonal antibody production, including very large scale, is produced exclusively *in vitro*.

Refinement to improve animal well being

- Investment in more comfortable spaces and surrounding environment surpassing legislative

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requirements was performed to improve animal well being;

- Development of a method to identify animal discomfort and to stop programme quickly.

II.2 QUALIFIED STAFF

Qualified and trained personnel are essential to provide all necessary care the animals deserve. Our staff members must follow a dedicated training prior to working in the facility areas. Additional compulsory trainings and continuous skill upgrading are performed. The whole staff experience is recorded. Staff training files contain the documentation of personal education, experience, skills and training for the position held.

II.3 COMPREHENSIVE TRACEABILITY

To ensure full traceability, all animals are identified and recorded in a register from their arrival to their departure. The identification system depends on the species. Most frequently, we use ear tag, fur coloration, cage label or chip. We also propose custom identification adapted to the customer needs while keeping in mind animal welfare.

According to our Quality Management System, highly qualified operators daily examine the animal state of health and report observations in dedicated sanitary sheets. On a quarterly basis, an external expert inspects animals and forwards his report to the director of the laboratory and to the competent authorities. At any time, experts can decide to audit our animal facility.

If any problem is reported with an animal, the responsible veterinarian is the first to be informed and provides the most adapted treatment. The director of the laboratory and the customer are then informed. The Sanitary sheet is updated. In case of emergency, responsible operators are trained to act immediately and limit animal suffering.

II.4 CAREFUL SELECTION OF ANIMAL ORIGIN

We attach a strong importance to the animals' quality and health and as such, we select our animals suppliers with the highest vigilance. Farm animals come from selected private producers while rodents are provided by accredited producers.

Rabbits are bred directly in dedicated areas of our animal facility by a dedicated staff. We continuously evaluate the most accurate needs for rabbits, and breeding is controlled accordingly to provide the exact number of animals. A circular system is in place to minimise inbreeding. Our management system is fully computerized, and data are archived for 10 years.

II.5 ADAPTED FEEDING

As animal feeding directly impacts animal welfare, we

guarantee that each animal is supplied with an appropriate balanced diet and we ensure quality of raw materials.

Feed is shipped by dedicated lorry and packaged in double-layer bags with a size adapted to an easy handling and appropriate workflow.

For each new feed batch, the supplier provides feed composition and disinfection certificate. Irradiation level indicated on each incoming bag is checked again before H_2O_2/peracetic acid (PAA) sanitization.

Feed intake is adapted to the animal species. The animal appetite and feed freshness are controlled 7 days a week.

11.6 METICULOUS SANITARY CONTROL

Sanitary aspects are extremely important for the good management of the animal facility and are consecutively highly controlled.

Sentinel animals

To guarantee healthy sanitary conditions and parallel to the daily observation, health status of sentinel animals are monitored every 3 month. Assays are performed in respect to the FELASA recommendations and depend on the controlled sentinel species. In order to get independent results, all assays are outsourced (Annexe 1).

Air quality and ventilation

Temperature, pressure, relative humidity and ventilation systems are recorded in real time. Ventilation is

configured to create a forced air circulation from the most sensitive areas to the less sensitive ones before being expelled outside. This pressure cascade aims at avoiding dust entry and microorganism contaminations.

Large animals rest in standard conditions with an outdoor airflow.

Specific Pathogen Free (SPF) animal housing room air is renewed 10-15 times per hour with 100% HEPA filtered air.

NH_3 and CO_2 are monitored on a regular basis.

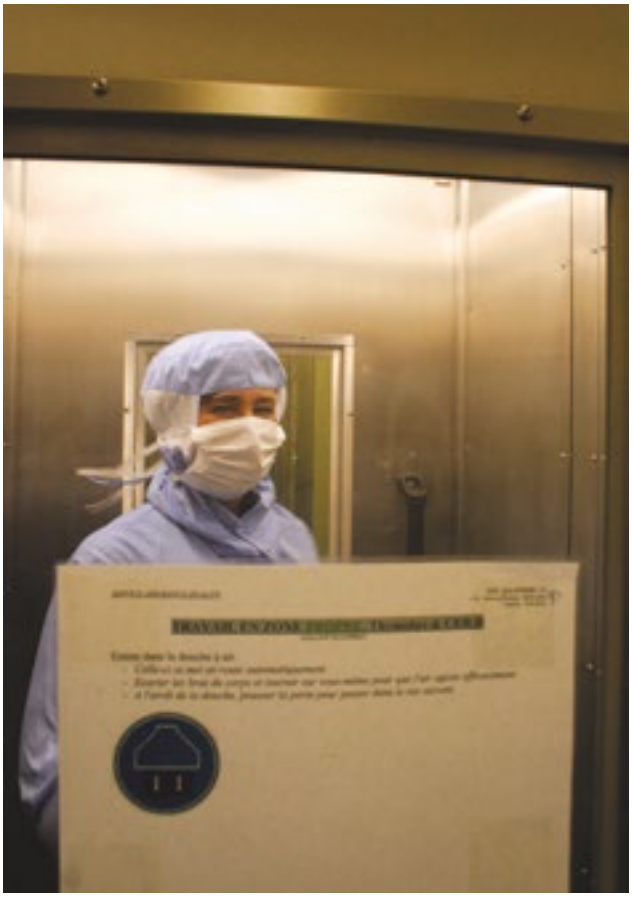
Should the threshold be exceeded, a call centre gives an instant warning, and adjustment is to be provided within 2 hours.

Decontamination and waste management

All incoming materials have to be carefully disinfected by a validated H_2O_2/PAA dry fog system. Good personal hygiene is mandatory. Drastic hygiene protocols (wet or air shower, hands washing with hydroalcoholic gel...) are required before entering any area of the animal facility. Traffic is always done from the cleanest to the dirtiest area, and cross contamination is prevented by a unilateral traffic pattern.

Cleaning

Animal observation is performed on a daily basis, including weekends and public holidays. Qualified staff ensures the cleanliness of the cages and pens, feed



and water availability and general animal welfare. Animal facility areas are cleaned 3-5 times a week with cleaning agents free from formaldehyde. The nature of the cleaning agent is changed every month in a 4-month cycle. According to ISO 9001 certification, all cleaning phases and observations are reported.

Grids, anti-rodent barriers, anti-flies and UV traps contribute to a healthy environment.

11.7 UNCOMPROMISED SAFETY

Barriers and magnetic doors protect the whole site. Each member of staff has its own access card to enter the building and to move from one area to another. Staff are present 24 hours a day. A computer system monitors all comings and goings.

An intrusion alarm system with a dialer ensures absolute security. Armoured doors and surveillance cameras protect the most vulnerable zones. □

Specialists are always

Need of more information?
Feel free to contact us
info@eurogentec.com

Antibody Services

available to you

With over 25 years of experience, we produce custom polyclonal and monoclonal antibodies with high flexibility. Polyclonal antibodies can be produced in mice, rats, guinea-pigs, rabbits, chickens and larger animals such as goats, pigs and llamas.

III. 1 HIGH EXPERTISE IN CUSTOM ANTIBODY PRODUCTION

During the whole animal immunisation and maintenance process, Eurogentec guarantees to respect the psychological and physiological well-being of animals. Antibody production is performed under ISO 9001 requirements.

To start an immunisation programme, the antigen can be injected by subcutaneous, intradermal, intramuscular,

intra-peritoneal or intravenous route. As an expert in antibody production, we offer the possibility to use the BL2 Antigen.

For every immunisation, we can develop customised programmes, or use the customer's own programme. We can manage many programmes simultaneously, and can easily adapt to high volumes.

III. 1 A CUSTOM POLYCLONAL ANTIBODIES

While we routinely perform the classical 3-month immunisation programmes, which uses Freund's complete/incomplete adjuvant, Eurogentec has developed the proprietary and highly efficient **Speedy 28-Day programme**, which uses an exclusive non-Freund's adjuvant protocol. Since its launch in 2007, we have performed more than 5000 Speedy 28-Day polyclonal programmes, with an unbeaten success rate. Many additional services are available, including Ab purification, labeling, coupling to magnetic beads and ELISA development.

Species	Housing capacity
rabbits	15.000
guinea pigs	**300***
hamsters	200*
rats	**200***
mice	400*
sheeps	**200***
goats	200*

Table 2 Capacities
for polyclonal antibody production.
*Can be easily adapted for larger customer's requests.

III. 1 B CUSTOM MONOCLONAL ANTIBODIES

Since 1996, Eurogentec has acquired a trusted experience in the field of custom monoclonal antibody production.
A classical monoclonal antibody programme is divided into 4 phases

With our step by step approach, you keep full control on the production process, which may be revised by you at any time. Our project management team keeps you informed in real time, and assists you throughout the whole process.

The production of monoclonal antibodies, starting from hybridomas obtained from our immunisation programme or provided by the customer, is typically performed *in vitro*. The ascites method is indeed widely discouraged due to the pain and discomfort caused to the animals and as such, it has been fully prohibited in Belgium since 2004. Accordingly, Eurogentec exclusively produces mAbs by the *in vitro* method with high expertise, and continuously improves its production processes to ensure the most reproducible and optimised production of high quality monoclonal antibodies from hybridomas.

Our facilities are adapted to produce from milligrams to grams of monoclonal antibodies. Based on the antibody amount requested and the clone productivity, we determine the best production scheme, using flasks, roller bottles or bioreactors.
By default, monoclonal antibodies are produced in DMEM medium supplemented with 10% fetal bovine

serum (FBS). They are purified on protein G matrix and lyophilized from 1mg/ml aliquots. Many additional services and options are however available on request.

III. 2 SPF ANIMALS FOR ACCURATE RESULTS

The quality of the produced antiserum depends not only on the animal physiology, but also on the animal housing and treatment conditions.

To reduce the initial background monitored prior to the immunisation, Specific Pathogen Free (SPF) animals can be useful.

Animals bred in SPF environment are free of some agents such as bacteria, parasites and viruses edited on the FELASA monitoring list. SPF areas are under positive air pressure compared to surrounding areas. Strict operational procedures and unidirectional traffic flows must be followed to avoid contact between clean and soiled supplies areas.

III. 3 AUTOPSY IN CASES OF DOUBT

In case of abnormal animal death, an autopsy can be performed on demand, internally or through an independent expert. Complementary bacterial or histological analyses can be performed by external partners.

Phase 1	
Immunisation of 4 mices	+/- 6 weeks
Phase 2	
Fusion/hybridoma Production	+/- 2-3 weeks
Phase 3	
Screening for positive hybridomas	+/- 4-5 weeks
Phase 4	
Cloning and isotyping of positive hybridomas	+/- 4-5 weeks

Because we want to make sure that you get the best solution that fits your needs, our specialists are available prior and throughout the whole project process to discuss and define with you the best production parameters.

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III. 4 FULL CONFIDENTIALITY

Eurogentec ensures that your project and your data will be handled under full confidentiality and will never be shared with any third party. Non-disclosure agreements can be executed.

Visitors must sign in advance a confidentiality clause and have to avoid within 3 days before the visit all contact with similar animal species to those housed.

During the visit, an employee always accompanies visitors who are identified by a dedicated I.D. badge.

Annexe 1

Sentinel Species

Species	Endpoint	Assay
MICE	*Clostridium piliformis*	IFA
	Minute mouse virus	ELISA
	Mouse hepatitis virus	ELISA
	Mouse parvovirus	ELISA
	Mouse rotavirus	ELISA
	Mycoplasma pulmoris	ELISA
	Pneumoniea virus of mice	ELISA
	Sendai virus	ELISA
	Theiler's murine encephalomyelitis v.	ELISA
	Citrobacter rodentium	Culture
	Pasteurellaceae	Culture
	Corynebacterium kutscheri	Culture
	Salmonella spp	Culture
	Streptococcus pneumoniea	Culture
	Streptococcus ß hemolytic	Culture
	Ectoparasites	Microscopy
	Endoparasites	Microscopy
	Gross Lesion	Necropsy
RATS	*Clostridium piliformis*	IFA
	Kilham rat virus	ELISA
	Mycoplasma pulmoris	ELISA
	Pneumoniea virus of mice	ELISA
	Rat parvovirus	ELISA
	Sendai virus	ELISA
	Sialodacryoadenitis virus	ELISA
	Toolan's H-1	ELISA
	Corynebacterium kutscheri	Culture
	Bordetella bronchiseptica	Culture
	Streptobacillus moniliformis	Culture
	Streptococcus ß hémolytic	Culture
	Streptococcus pneumoniea	Culture
	Pasteurellaceae	Culture
	Salmonella spp	Culture
	Ectoparasites	Microscopy
	Endoparasites	Microscopy
	Gross Lesion	Necropsy

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Annexe 1

Sentinel Species

Species	Endpoint	Assay
HAMSTERS	*Clostridium piliformis*	IFA
	Lymphogenic choriomeningitis virus	ELISA
	Sendai virus	ELISA
	Pasteurellaceae	Culture
	Salmonella spp	Culture
	Ectoparasites	Microscopy
	Endoparasites	Microscopy
	Gross Lesion	Necropsy
GUINEA PIGS	*Chlamydia psittaci*	IFA
	Encephalitozoon cuniculi	IFA
	Guinea pig adenovirus K87	ELISA
	Guinea pig adenovirus FL	ELISA
	Sendai virus	ELISA
	Bordetella bronchiseptica	Culture
	Dermatophytes	Culture
	Corynebacterium kutscheri	Culture
	Streptobacillus moniliformis	Culture
	Streptococcus pneumoniea	Culture
	Streptococcus ß hemolytic	Culture
	Yersinia enterocolitica	Culture
	Pasteurellaceae	Culture
	Salmonella spp	Culture
	Ectoparasites	Microscopy
	Endoparasites	Microscopy
	Gross Lesion	Necropsy

Annexe 1

Sentinel Species

Species	Endpoint	Assay
RABBIT	*Clostridium piliformis*	IFA
	Encephalitozoon cuniculi	IFA
	Pneumoniea virus of mice	ELISA
	Rabbit rotavirus	ELISA
	Rabbit pox virus	ELISA
	RHDV	ELISA
	Reovirus type 3	ELISA
	Sendai virus	ELISA
	Simian virus 5	ELISA
	Toxoplasma gondii	ELISA
	Bordetella bronchiseptica	Culture
	Corynebacterium kutscheri	Culture
	Dermatophytes	Culture
	Pasteurella multocida	Culture
	Salmonella spp	Culture
	Ectoparasites	Microscopy
	Endoparasites	Microscopy
	Gross Lesion	Necropsy

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Because we want to make sure you get the best solution, our specialists are available prior and throughout the whole project process. Feel free to contact us. Together we will discuss and define the best strategy your application requires.

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Contact

Do not hesitate to contact us to start discussing your project.
We will define together the best procedure adapted to your needs.

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