



Research Models
and Services

Health monitoring

The disease status of laboratory animals has improved significantly over the last 25 years. This has resulted in the increased availability of animals free from the major pathogens, that are robust under experimental procedures and that do not represent a hazard to other animals in the facility. At the same time, the increase in the use of genetically modified animals and their movement from diverse sources throughout the world has presented a serious threat to animal health and has resulted in the breakdown of some animal facilities with serious infections.

The maintenance of health status of the animals within the facility, whilst fulfilling a research requirement for specialized laboratory animals, presents a difficult challenge. A program of health monitoring is therefore essential, in order to assure managers and researchers that the standard of animal health within the facility is maintained and that experiments will not be compromised by unexpected infections.

Outbreaks of infectious diseases occur from time to time. Many of these infections are latent and although they do not always lead to clinical symptoms, they can have an impact on the outcome of animal experiments. Some micro organisms can even infect humans (zoonoses). These are a number of reasons for every institute to set up a health surveillance program.

The Envigo advantage

Custom made testing profiles and health surveillance programs

- + Turnaround time serological results < 48 hours
- + Customers pay for the result – not the retesting
- + Regular pick up services of animals
- + Discuss interpretation of positive results with our in-house experts

Envigo offers its clients numerous possibilities in setting up these programs including testing profiles, frequencies of testing, number of animals to be monitored etc. Our ISO 9001-2008 certified diagnostic laboratories are located in the UK and Italy and include highly qualified microbiologists, (molecular) biologists and veterinarians.

The routine testing of laboratory animals includes the use of serology, bacteriology, and parasitology which is usually undertaken on live animals, which can be picked up from your facility by dedicated Envigo trucks. Alternatively samples can be sent by overnight courier.

Serology

Envigo is testing serum from animals for the presence of antibodies to viruses, mycoplasma and some bacteria and parasites. The primary test nowadays for most agents is the Microbead Assay. An antigen, such as a virus, that is coated onto the beads will react with its specific antibody if present in the serum of the animal / sample tested. This is a very sensitive and specific method which requires only 2 µl of diluted, non-haemolysed serum. A detailed leaflet on this technology is available on request.

The Enzyme Linked Immunosorbent Assay (ELISA), once the primary serological test, is now used only for those agents for which there is no Bead Assay alternative. It is now a secondary method for the majority of tests, together with indirect Immunofluorescence Assay (IFA).



Serological testing detects the presence of specific antibody against the agent being tested. Therefore it is regarded as an indirect test, which can provide controversial results. If a sample gives a positive or borderline (equivocal) result, it is retested using the primary method. This is then supported by the other methodologies, ELISA and IFA. Additionally, in some cases (for example parvoviruses) PCR can also be used. We may also use secondary laboratories, if it is felt that a second opinion is appropriate. Note that you only pay for the result, not for the additional testing.

Dried blood spot technology

Envigo, in keeping with the promotion of the 3Rs philosophy, are now accepting dried blood spot (DBS) cards as an alternative sample material to traditional blood serum for serological testing. DBS provides a simple method of sample collection, shipping, and storage of blood samples for serological testing. Due to the extremely low blood volume required, samples can be obtained from live animals. The simplicity of DBS means that the use of specialised instruments, such as centrifuges, pipettes or vials is not required for sampling purposes. There are also no limitations in the serological tests available for DBS samples; all serological assays available for testing via the use of serum are also available for DBS samples.

Envigo supplies all the necessary items required for sampling and shipping of the DBS samples, please enquire with your local Envigo sales representative for further details. A detailed leaflet on DBS sampling and shipping is available on request.

Bacteriology

In general, apart from those that are detected using serological techniques, bacteria are cultured using samples taken from specific sites from the animal. For example, a routine microbiological analysis consists of taking swabs from the nasopharynx and caecum in order to isolate respiratory and enteric pathogens respectively.

The swabs are plated onto various selective and non-selective media. Selective media are used where organisms are considered fastidious in their growth requirements or may easily be overgrown and therefore hidden by other bacterial colonies. A commonly used non-selective medium is blood agar. This medium is chosen as most bacteria will grow readily on it.



Once a primary culture has been obtained, the various colonies are sub cultured onto individual agar plates in order to obtain pure cultures, which can then be identified. Vitek2 and API strips are used for further identification of bacterial colonies.

Antibiotic sensitivity testing (AST) can be performed on a variety of bacterial species, after a positive identification, ensuring prompt and efficient use of medication within and animal colony.

Parasitology

The animals, once euthanized, are examined for parasites. Ectoparasites can usually be readily detected behind the ears, around the nose and eyes, or behind the neck. Endoparasites are best detected by examining wet preparations of the large and small intestines, whilst taking account of any lesions that may be apparent in other tissues and organs, e.g. the liver, which may signal infestation by parasites. Examinations are made using a microscope for protozoa, worms, cysts, eggs and oocysts (of coccidia).

False positives in parasitology are rare. This is a direct test, and the parasite can be observed either by the naked eye or through a microscope. In our labs, all positive observations must be verified by a second individual, who countersigns the report. In addition, photographs and videos are taken via the microscope to allow for further verification or reference at a later date.

Molecular biology

During the last decade, molecular biological tests have played an increasing role in the detection of infectious agents. Our diagnostic laboratories historically have utilized the Polymerase Chain Reaction (PCR) to test for some microorganisms, e.g. *Helicobacter* spp, and *Pneumocystis carinii*.

As part of our commitment to continuous improvement in animal welfare and promotion of the 3Rs, Envigo is expanding its use of Real Time PCR technology in monitoring animal health.

PCR testing improves specificity and sensitivity and allows for pooling samples (e.g. screening of IVC racks) and does not require the sacrifice of the animal itself. Envigo has designed a tracking panel of the most prevalent infectious agents as a useful and economical means of monitoring valuable laboratory mouse colonies as part of a more extensive health monitoring program. The panel includes Mouse Hepatitis Virus (MHV), Parvovirus, Murine Norovirus (MNV), *Pasteurella pneumotropica* and pinworms (*Syphacia obvelata*, *S. muris*, *Aspiculuris tetraptera*).

PCR testing is also used as an alternative test method in case confirmation of positive findings for organisms is required.

Envigo is expanding its use of Real Time PCR technology to provide additional assays and supplement existing assays to progressively expand our health monitoring programs. We can also assist you in coordinating Real Time PCR testing of your animals or samples.

Agent listing

SEROLOGY	TEST METHOD	MOUSE	RAT	HAMSTER	GUINEA PIG	RABBIT
Ectromelia	Bead / ELISA	√				
Guinea Pig Adenovirus (GpAd)	ELISA				√	
Guinea Pig Cytomegalovirus (GpCMV)	IFA				√	
Guinea Pig Parainfluenza Virus 3 (PI-3)	ELISA				√	
Hantaan Virus	Bead / ELISA	•	√			
Kilham Rat Virus (KRV)	ELISA		√			
Lactic Dehydrogenase-Elevating Virus (LDEV)	ENZYME	•				
Lymphocytic Choriomeningitis Virus (LCM)	Bead / ELISA	√		√		
Minute Virus of Mice (MVM)	Bead / ELISA	√				
Mouse Adenovirus type 1 (Mad-FL)	Bead / ELISA	√	√			
Mouse Adenovirus type 2 (Mad-K87)	Bead / ELISA	√	√			
Mouse Cytomegalovirus (MCMV)	Bead / ELISA	•				
Mouse Hepatitis Virus (MHV)	Bead / ELISA / PCR	√				
Murine Noro Virus (MNV)	Bead / ELISA / PCR	√				
Mouse K-virus	ELISA	•				
Mouse Parvo Virus (MPV - NS-1)	Bead / ELISA / PCR	√				
Mouse Rotavirus (EDIM)	Bead / ELISA	√				
Mouse Thymic Virus (MTV)	IFA	•				
Pneumonia Virus of Mice (PVM)	Bead / ELISA	√	√	•		
Polyoma virus	ELISA	•		•		
Rabbit Adeno Virus	ELISA					•
Rabbit Corona Virus	ELISA					•
Rabbit Haemorrhagic Disease Virus (RHDV)	ELISA					√
Rabbit Pox Virus (Myxomatosis)	ELISA					•
Rabbit Rotavirus	ELISA					√
Rat Minute Virus (RMV)	ELISA		√			
Rat Parvo Virus (RPV)	ELISA		√			
Rat Theilo Virus (RTV)	ELISA		√			
Reovirus Type 3 (Reo 3)	Bead / ELISA	√	√			
Sendai	Bead / ELISA	√	√	√	√	
Sialodacryoadenitis / Rat Coronavirus (SDAV)	ELISA		√			
Theiler's Murine Encephalomyelitis Virus (GD VII)	Bead / ELISA	√				
Toolan H1	ELISA		√			

Pathology

Tissues and organs are taken if lesions are observed at routine necropsy. They can be submitted for histopathology provided the customer wishes, as it will include extra charges. Samples will then be submitted for further evaluation.

Testing profiles

It has to be accepted that any health program is a compromise between what is practicable and affordable to achieve on the one hand, and the significance of the results on the other. Although the FELASA Recommendations are not a requirement that animals tested are free from all organisms listed, these recommendations are often used as a reference for setting up comprehensive health programs in lab animal facilities. Microbiological standardisation is an important tool for reproducible animal experiments.

Above and on the next page you will find the listing of organisms that can be taken into account when setting up your surveillance programs for rodents and rabbits. On demand agents can always be added to fit your requirements.

Sentinels

There are some instances when the use of sentinel animals is recommended. Sentinel animals are those that are introduced to the main population specifically for the purpose of health monitoring, when it is difficult or inappropriate to sample animals from the main population. One example of such a case is when using racks of individually ventilated cages (IVC's), where it may not be practicable to screen animals in every cage on the rack. Another example is monitoring immunodeficient or immunocompromised animals, where serology is inappropriate.

BACTERIOLOGY

	TEST METHOD	MOUSE	RAT	HAMSTER	GUINEA PIG	RABBIT
<i>Bordetella bronchiseptica</i>	Culture	•	•		√	√
CAR bacillus	ELISA	•	√		•	√
<i>Citrobacter rodentium</i>	Culture	√				
<i>Chlamydomphila caviae</i>	IFA				•	
<i>Clostridium piliforme</i> (Tyzzer's disease)	Bead / ELISA	√	√	√	√	√
<i>Corynebacterium bovis</i> (HAC)	PCR	•	•			
<i>Corynebacterium kutscheri</i>	Culture	√	•	√	√	
Dermatophytes	Culture				•	•
<i>Escherichia coli</i> (enteropathogenic strains)	Culture					•
<i>Helicobacter</i> spp.	PCR	√	√	√		
<i>Klebsiella pneumoniae</i>	Culture	•	•			
<i>Klebsiella oxytoca</i>	Culture	•	•			
<i>Mycoplasma pulmonis</i>	Bead / ELISA	√	√			
Pasteurellaceae	Culture	•	•	•	•	•
<i>Pasteurella multocida</i>	Culture					√
<i>Pasteurella pneumotropica</i>	Culture / PCR	√	√	√		
<i>Pneumocystis carinii</i>	PCR		√			
<i>Pneumocystis murina</i>	PCR	•				
<i>Pneumocystis oryctolagi</i>	PCR					•
<i>Pseudomonas aeruginosa</i>	Culture	•	•		•	
<i>Proteus</i> spp.	Culture	•	•			
<i>Salmonella</i> spp.	Culture	√	√	√	√	√
<i>Staphylococcus aureus</i>	Culture	•	•		•	•
<i>Streptobacillus moniliformis</i>	Culture	√	√		√	
Streptococci Beta-haemolytic (not Group D)	Culture	√	√		√	
<i>Streptococcus pneumoniae</i>	Culture	√	√		√	
<i>Yersinia pseudotuberculosis</i>	Culture				•	
<i>Treponema paraluisicuniculi</i>	IFA					•

PARASITOLOGY

	TEST METHOD	MOUSE	RAT	HAMSTER	GUINEA PIG	RABBIT
Ectoparasites	Micr.	√	√	√	√	√
Endoparasites	Micr.	√	√	√	√	√
Pinworms	Micr. / PCR	√	√	√	√	√
<i>Encephalitozoon cuniculi</i>	ELISA	•	•	•	√	√

√ - Agents recommended by FELASA 2014

• - Optional agents for specific needs, e.g. in case of immunodeficient mice and rats or to include opportunistic bacteria (so called FELASA Plus / SOPF quality)

It is advised that sentinels are sufficiently exposed to the animal colonies (at least 6 weeks), and that animals are of such an age, that they are capable to have an adequate immune response when they reach the lab (> 8 weeks). Sentinel animals should be selected for immune responsiveness, susceptibility to pathogens of interest (and therefore negative for these organisms!), and ease of identification.

Isolator bred and maintained sentinels from Envigo are at minimum free of opportunistic bacteria (often referred as FELASA Plus or SOPF quality).

Sample size and frequency

It should be obvious that the more frequent the sampling and the bigger the sample size, the greater the confidence in the information obtained from the health monitoring program. However, sampling frequency and sample size must be balanced against economic constraints and the availability of animals.

Factors that influence these are:

- + Current microbiological status of the animals and type of housing (eg IVC versus barrier)
- + Level of risk of contamination from other nearby populations
- + Facility design and environment
- + Frequency of animal, biological material and personnel population changes
- + Importance of a pathogen or other contaminant to the use of the population in research protocols
- + Economic considerations



It should be recognised that although the sample size required to detect a single agent can be determined with reasonable precision, it is virtually impossible to maintain the same degree of precision for all agents to be included in a large test battery. Different agents have typically different infection rates within animal colonies

Attention should also be given to sampling animals of both sexes, different ages and if possible different types of stocks, as some stocks are more / less sensitive to pathogenic agents.

From the above you can learn the complexity of establishing the right health program. Envigo has the in-house experience and expertise to help customers design a health monitoring program to suit their individual needs.

Reporting

The reporting system Envigo uses is LIMS, which is an acronym for Laboratory Information Management System. This is a *bench-based* system which is designed around the technician entering raw data directly into the system. Each submission receives a reference number (e.g. 15-3856) which covers all of the samples received within that submission. Each sample is given an individual number (bar-coding) so that each sample can be tracked individually. The reference and sample numbers follow the samples through the laboratory processes through to the final report.

At any one time, a health screen must be regarded as a "snap-shot" of the microbiological status of the colony at the time of sampling. As a consequence, building up historical data, by microbiological unit, is very important for understanding the presence of organisms (esp. pathogens) in your facility.

A full report is normally provided within 10 working days from receipt of animals. If necessary, serology data can already be provided within 48 hours after receipt of the animals or samples. The LIMS reports can provide historical data for individual units and areas, if required.

Interpretation of results

Establishing a health monitoring program is one thing, but quite another to handle the results and the questions that may arise. Some of these questions that could arise:

- + Why do only some of the animals submitted test positive on occasion for certain agents, and not all animals?
- + Why do test results for different submissions from the same area vary from time to time?
- + How reliable are the test results? What should be done if an unexpected positive result is obtained?
- + Do test results vary between different test methods and between different laboratories? If so, why?

Serology results could for example be affected by haemolysis of the serum sample, repeated freezing / thawing as well as prolonged storage in freezer, over-dilution, heat- inactivation, age of the animals sampled and settling effect of antibody in

serum samples. The presence of antibody, bacteria or parasites will also depend upon the length of time the animal has been exposed to the agent.

Envigo has developed a comprehensive document on the interpretation of testing results, which is available on request.

In case colonies are confirmed to be positive for pathogenic organisms, Envigo can assist in cleaning up the colonies through rederivation (embryo transfer and / or caesarean section). In case of positive endoparasite findings (such as pinworm infections) we can offer you treatment schedules, such as use of Fenbendazol in diet.

Testing of biological materials

Biological materials such as tumours, hybridomas, cell lines and blood products can be contaminated when they are sourced from infected animals. Introduction of these materials into the animal unit is therefore subject to risks. In the past antibody production tests were used in mice (MAP) or rats (RAP). These tests have been largely replaced by molecular biological techniques (PCR), which provide faster results without the need for experimental animals. This infectious microbe PCR amplification test (IMPACT) is conducted at UMRADIL. Instructions and guidelines can be provided by our technical team.

Transport

Preferably live animals should be sent to our laboratories. Therefore Envigo can arrange collection of the animals from your facility for screening, on our dedicated vehicles. We do not transport animals for screening with animals for delivery to customers, as we are aware of the possible contamination risk. Our vehicles are fumigated once screening animals have been delivered to our laboratories.

For packing of the animals to be screened, Envigo offers filtered boxes in different sizes, incl. gel so animals arrive in optimal condition at our laboratories. Information on all pick up services can be obtained from your local Customer Service Department.

Apart from sending live animals, it's also possible to send samples (sera, faecal samples etc.) at ambient temperature by overnight courier. Otherwise it is recommended that the shipment is sent on cool packs or dry ice. At any time the samples should be packed in leak-proof containers, surrounded by absorbent material.

Useful references

- 1) FELASA Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units 2014 (*Laboratory Animals Mähler et al*)
- 2) Natural Pathogens of Laboratory Animals, their effects on research (2003), David G.Baker

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