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Risks of infection from biological materials

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Author: Werner Nicklas, DKFZ Heidelberg

How are infectious agents introduced into an animal facility?

In order to keep laboratory animal colonies and units, especially of rodents, free from unwanted microorganisms, all potential sources of infection must be identified. There is no doubt that infected animals represent the highest risk. All biological materials originating from such animals (e.g. serum, ascitic fluid, tumours, organ explants, cells, fertilized eggs, embryos, sperm) may also be contaminated if they have been taken from an infected organism. Such materials must, therefore, also be considered as possible sources of infection. Even samples from human origin may be contaminated by rodent microorganisms after animal passages. The documentation about the history of biological materials, even if they originate from commercial vendors or from culture collections, is frequently fragmentary. It is therefore advisable to test such samples for contamination before use.

Which agents may be introduced?

Viruses are frequently transmitted by biological materials, but also bacteria (e.g., *Pasteurella pneumotropica,* (Simpson et al., 1980), *Helicobacter hepaticus* (Goto et al., 2001) and others (Criley et al., 2001) as well as parasites (*Encephalitozoon cuniculi* (Petri, 1965)) have been detected as contaminants. Some murine viruses, like minute virus of mice (MVM), K virus, Theiler’s murine encephalomyelitis virus and mouse adenovirus, were first isolated from contaminated virus pools. Polyoma virus, mouse parvovirus (MPV), Kilham rat virus (KRV) and Toolan's H-l virus were found originally in contaminated tumours or cells. The most recently published outbreaks of ectromelia in laboratory mice caused by contaminated sera also underline the immense risk of agent transmission by biological materials (Dick et al., 1996, Lipman et al., 2000b, Labelle et al., 2009).

A risk of infection exists also for humans. For example, the lymphocytic choriomeningitis virus (LCMV) (Bhatt et al., 1986, Dykewicz et al., 1992) and hantaviruses (Yamanishi et al., 1983) have been found in rodent tumours. Reports of human infections caused by contact

with biological materials exist for both viruses (Biggar et al., 1977, Bowen et al., 1975, Kawamata et al., 1987). There is a potential risk for infection in humans when handling as well as using biological material therapeutically (e.g. monoclonal antibodies) (Carthew, 1986, Harbour and Woodhouse, 1990). Therefore, all biological material must be tested for viruses before use.

Storage of contaminated biological material at low temperatures (deep freezing) does not reduce infectivity. Therefore, long-stored samples can be hazardous and may represent a serious health risk for animals and humans. Other agents, despite the lack of clinical symptoms in animals or humans, can still influence the results of animal experiments, leading to misinterpretations and to the need to repeat experiments (Peterson, 2008). Examples of this are parvoviruses [e.g., MVM, MPV, KRV, rat minute virus (RMV)] (Guetta et al., 1986, Moody et al., 2011) and, not to be forgotten, lactate dehydrogenase elevating virus (LDV) (Riley, 1974). LDV is a frequent contaminant of biological material originating from mice. Published data show that LDV can be present in a high percentage (up to 70%) of transplantable tumours (Collins and Parker, 1972, Nicklas et al., 1993). As this virus causes a lifelong viraemia, inevitably all material originating from LDV-infected mice is contaminated with the virus.

Can infectious agents be eliminated from contaminated samples?

In general, it may be possible to decontaminate biological material contaminated with viruses. The choice of procedure strongly depends upon the material itself and upon the virus involved. In case of cell-free samples, i.e. serum or ascitic fluid, physical or biochemical procedures are often suitable to render the material virus- or agent-free. Cellular material like transplantable tumours may be suitable for sanitation by transplantation in a host species which is refractory to the contaminating virus (Rülicke et al., 1991, Dagnaes-Hansen and Horsman, 2005, Takakura et al., 2000, Nakai et al., 2000). In the case of LDV, *in vitro* cultivation of contaminated cells is the most reliable method for elimination of this virus (Plagemann and Swim, 1966); other methods have also been published (Liu et al., 2011). In many cases, however, the elimination of an agent will not be possible, or will cause considerable effort and costs.

How can biological materials be tested for contamination?

Prevention and screening methods for early diagnosis of contamination are thus of high priority and importance. It is strongly advised that materials of animal origin, which bear a potential risk of infection, should be monitored for contamination prior to use in animal experiments. Material of human origin which may be contaminated should be handled similarly. Only biological material which has been proven to be free of infectious agents should be used. Testing is recommended if a certificate of harmlessness is not available for a sample or for a defined batch.

The so-called mouse/rat antibody production test (MAP/RAP-test) has been used for decades to detect or exclude contamination by infectious agents (viruses, bacteria,

parasites) (Collins and Parker, 1972, Nicklas et al., 1993, Lewis and Clayton, 1971). This test relies on the production of antibodies against infectious agents contaminating a sample. The material to be tested is injected into agent- and antibody-free animals, and 3 to 4 weeks later blood samples from these animals are examined for antibodies against likely agents.

Several methods exist in addition to the MAP/RAP-test to screen for contamination. These are, e.g., cell culture techniques (Desouza and Smith, 1989) and molecular methods, especially polymerase chain reaction (PCR) (Bauer et al., 2004, Blank et al., 2004, Bootz and Sieber, 2002, Bootz et al., 2003, Morse, 1990, Yagami et al., 1995, Bootz and Wolf, 2007). Agent detection or exclusion by PCR is cheaper and faster to conduct as MAP testing. In addition, use of live animals is not necessary. However, these methods are not yet generally established, and MAP/RAP-testing may in specific cases be superior to PCR (Lipman et al., 2000a). In addition, PCR does not provide information about the infectivity of an agent contaminating a sample because both active and inactivated agents are detected. Tests for bacterial contamination can easily be conducted by traditional culture techniques. Exclusion of human pathogens from samples of human origin (e.g., hepatitis viruses, HIV) should be self-evident.

Mycoplasma species most commonly found in cell cultures (including ES cells) are primarily of bovine, porcine or human origin. They are in most cases apathogenic for laboratory animals (mouse, rat) and are usually eliminated by macrophages during animal passages, even in immunodeficient animals like nude mice. However, Mycoplasma species infecting rodents (e.g., *M. pulmonis*) have also been detected after *in vitro*-passages of cells (Nicklas et al., 1993). But also non-rodent mycoplasma species should not be tolerated as they can influence numerous cell functions and may have impact on animals or manipulations with animals (e.g., breeding efficiency) (Markoullis et al., 2009, Boslett et al., 2014). Mycoplasma detection is best conducted by PCR.

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