Microbiological Control of Murine Viruses in Biological Materials: Methodology and Comparative Sensitivity A review

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Summary
Introduction of microbiologically contaminated materials into mice can cause infections and distort research results. As counter measures, biological materials should be routinely screened prior to use, ideally by specific, sensitive, and reliable diagnostic methods. In this overview, we present a description of the currently used detection methods for murine viruses, including virus isolation in cell culture, the mouse antibody production (MAP) test, infant mouse bioassay and the PCR, that are relevant to routine health monitoring and summarize available data on their comparative sensitivities. Current diagnostic trends toward increased use of *in vitro* methods, apart from providing sensitive and specific results, contribute to refinement, replacement and reduction of the use of laboratory animals.

Introduction
Globalization of biomedical research has expanded the exchange of murine biological materials as well as mice thereby also increasing the risk of inadvertently transferring infectious microorganisms to mice and, hence, mouse colonies *(Nicklas et al., 1993; Nicklas and Weiss, 2000)*. Biological materials employed in mouse-related research include embryos, sera, monoclonal antibodies, ascitic fluids, cell lines, cell culture products, tissues and transplantable tumours. They often originate from mice but also can be derived from other species including rat, human, hamster and rabbit *(Collins and Parker, 1972; Shek, 1987; Nicklas et al., 1993; Yagami et al., 1995; Nakai et al., 2000)*. Primary materials that are free of murine pathogens may become contaminated during passage or propagation in infected mice. Additionally, the microbiological status of materials obtained prior to regular health monitoring of mouse facilities is often unknown. Therefore, biological materials should be tested for contaminants, especially prior to use *in vivo* by sensitive and reliable diagnostic methods.
The mouse antibody production (MAP) test *(Rowe et al., 1959)* has been traditionally a widely used screening method. It is based on the detection of antibodies against murine viruses after inoculation of mice with biological samples of interest. The infant mouse bioassay is a less widely used method and entails inoculation of neonatal mice with a given specimen to assess any virus-related pathogenic effects. By contrast, the most popular *in vitro* methods are virus isolation in cell culture and the polymerase chain reaction (PCR) to detect viral DNA or RNA in biological materials. This overview gives a description of the methods and compares and contrasts their sensitivities. It constitutes a valuable and quick reference for routine health monitoring of mice and microbiological examination of biological materials as well as the aid in the evaluation of experimental data.

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Diagnostic methods for murine viruses

In this overview, we discuss methods that are primarily qualitative, that is, virus isolation in cell culture, MAP test, the infant mouse bioassay and the gel-based PCR. As a quantitative method, the real-time PCR is also described. The relevant mouse viruses are listed in Table 1. Sensitivity, in this context, refers to the smallest amount of analyte that can be distinguished from background.

Virus isolation in cell culture

Table 1 lists cell lines used to propagate and/or isolate murine viruses, their origin and the interval after inoculation required to observe a cytopathic effect (CPE). Note that cell lines permissive for murine viruses are not exclusively of mouse origin. They can also originate from monkeys, hamsters or even humans (Table 1). Some cell lines such as L929 and BHK-21 are permissive to many viruses whereas others such as NCTC-1469 are more virus-specific. The selection of cell lines for virus detection should account for the fact that lines conducive to virus propagation may not develop overt CPEs. For example, some strains of mouse hepatitis virus (MHV) are propagated in NCTC-1469 cells but virus isolation is often performed in L929 *(Mahabir et al., 2004)*. The onset and severity of CPEs can vary with virus strain. As shown in Table 1, these characteristics determine the endpoint of virus detection in cell culture, which can be 24 hours for some agents such as some MHV strains on L929 cells or 14 days in the case of polyoma virus on mouse fibroblasts.

Virus isolation takes longer than PCR analysis but is generally faster than the MAP test. Specimens containing small quantities of virus may require subculture of inoculated cultures before CPEs can be detected *(Garnick, 1996)*. This potential delay can be reduced by the simultaneous inoculation of several permissive cell lines. The presence of viral antigens may be detected earlier by immunostaining of inoculated cultures with panels of virus-specific antisera. It should be noted that some viruses such as K virus, Sendai virus, lactic dehydrogenase virus (LDV), mouse thymic virus (MTV) and enterotropic MHV strains do not replicate well in cell cultures *per se* and even where virus replication does occur, not all induce CPEs.

Although virus isolation in cell culture spares the use of live mice, the above-mentioned disadvantages, its relatively low sensitivity, and the requirement for highly-trained staff render this method unattractive for routine diagnostic work. Thus, it is most relevant when other detection methods fail or when attempting to isolate viruses from previously unrecognized diseases *(Wobus et al. 2004; Hsu et al. 2005)*.

MAP test

In the MAP test, first developed in 1959 for polyoma virus *(Rowe et al., 1959)*, seronegative, immunocompetent adult mice are inoculated with the test material. If viruses are present in an immunogenic dose antibodies are produced. Inoculation routes are intranasal, oral and intraperitoneal. To improve the effectiveness of this assay, these routes are combined. In general, outbred strains of mice are inoculated with the original and a tenfold dilution of the test material or with the diluent in controls. Tissue or cell culture specimens contain routinely 10^6 to 10^7 cells/ml diluent to maximize the sensitivity *(Nicklas et al., 1993; Nakai et al., 2000; Bootz et al., 2003; Mahabir et al., 2004)*. Standard MAP testing of pharmaceutical products requires inoculation of five mice each with 0.5 ml intraperitoneally, 0.05 ml intranasally and 0.05 ml orally. Inoculated mice are kept in an environment that precludes the entry and exit of pathogens.

A variation of the MAP test is used to assay for LDV in which inoculated mice are tested for elevations in lactic dehydrogenase (LDH) 3-5 days post inoculation and after 4 weeks for seroconversion to other viruses *(Compton and Riley, 2001)*.

Although established some 50 years ago the MAP test is still used for routine screening of biological materials. In fact, the United States Food and Drug Administration *(FDA, 1998)* recommends that a MAP test should be performed on any master cell

Table 1. Murine viruses relevant for screening of biological materials and a summary of typical cell lines used for their propagation and/or isolation

Virus	Virus family	Genome	Cell line (Reference)	Cell type	CPE + (days) *
Mouse adenovirus (Mad)	Adenovirus	DNA	L929 (Bootz et al., 2003)	mouse fibroblasts	3
			CMT-93 (Smith et al., 1986)	mouse rectal carcinoma cells	3
Mouse hepatitis virus (MHV)	Coronavirus	RNA	NCTC-1469 (Jacoby and Fox, 1984)	mouse fibroblasts	several
			L929 (MHV-3) (Bootz et al., 2003; Jacoby and Fox, 1984)	mouse fibroblasts	1
Mouse K Virus (K virus)	Papovavirus	DNA	n. a. (passaged in mice)	n. a.	none
Mouse rotavirus (EDIM)	Reovirus	RNA	MA-104 (Bootz et al., 2003; Jacoby and Fox, 1984)	rhesus monkey kidney cells	1
Mouse parvovirus (MPV)	Parvovirus	DNA	CTLL-2 (Besselsen et al., 2000)	murine cytotoxic T cells	n. a.
			L3 (McKisic et al., 1993)	murine cytotoxic T cells	n. a.
Mouse minute virus (MMV)	Parvovirus	DNA	L929 (Bootz et al., 2003; Jacoby and Fox, 1984)	mouse fibroblasts	7
			C-6 (Jacoby and Fox, 1984)	rat glial cells	n. a.
			EL-4 (Bonnard et al., 1976)	murine lymphoma	3-4
Pneumonia virus of mice (PVM)	Paramyxovirus	RNA	BHK-21 (Bootz et al., 2003; Jacoby and Fox, 1984)	baby hamster kidney cells	7
Reovirus type 3 (Reo3)	Reovirus	RNA	BHK-21 (Bootz et al., 2003; Jacoby and Fox, 1984)	baby hamster kidney cells	5
			L (Jacoby and Fox, 1984)	mouse fibroblasts	n. a.
Sendai virus	Paramyxovirus	RNA	n. a. (chick embryo)	n. a.	none [a]
Theiler's murine encephalomyelitis virus (TMEV)	Picornavirus	RNA	BHK-21 (Bootz et al., 2003; Jacoby and Fox, 1984)	baby hamster kidney cells	1
Ectromelia virus	Orthopoxvirus	DNA	HeLa (Bootz et al., 2003; Jacoby and Fox, 1984)	human cervix carcinoma cells	3
			B-SC-1 (Jacoby and Fox, 1984)	african green monkey kidney cells	n. a.
Hantaviruses	Hantavirus	RNA	Vero E-6 (McCormick 1982, Aitichou 2005)	african green monkey kidney cells	none
			A549 (French et al., 1981)	human lung cancer cells	none
Lactic dehydrogenase virus (LDV)	Togavirus	RNA	n. a. (passaged in mice)	n. a.	none
Lymphocytic choriomeningitis virus (LCMV)	Arenavirus	RNA	L929 (Bootz et al., 2003; Jacoby and Fox, 1984)	mouse fibroblasts	4
			BHK-21 (Jacoby and Fox, 1984)	baby hamster kidney cells	n. a.
Mouse cytomegalovirus (MCMV)	Herpesvirus	DNA	MEF (Bootz et al., 2003; Jacoby and Fox, 1984)	mouse embryo fibroblasts	6
Mouse Norovirus (MNV-1)	Calicivirus	RNA	RAW 264.7 (Wobus et al. 2004, Hsu et al. 2005)	murine macrophage cells	36-48h
Mouse polyoma virus	Papovavirus	DNA	MEF (Bootz et al., 2003; Jacoby and Fox, 1984)	mouse embryo fibroblasts	10
Mouse thymic virus (MTV)	Herpesvirus	DNA	n. a. (passaged in newborn mice)	n. a.	none

+: CPE: cytopathic effect, *: days of incubation to CPE, n. a.: not available, [a] : allantoic fluid agglutinates chicken red blood cells

bank and end-of-production cells derived from murine cell lines and on all lots of monoclonal antibodies (mAb) derived from mouse ascitic fluids. The list of viruses targeted by the FDA includes all those described in Table 1 except mouse parvovirus (MPV) and mouse norovirus (MNV-1). Furthermore, the FDA *(1997)* specifies that materials contaminated with lymphocytic choriomeningitis virus (LCMV), Reo3, Sendai virus or Hantaan virus should not be used for mAb production due to their zoonotic nature.

The major advantage of the MAP test is that multiple viruses can be detected simultaneously by serological assay. In addition, it may reveal previously unknown viruses if mouse inoculation produces unanticipated clinical signs or lesions. A major disadvantage of the MAP test is that false-positive results may occur due to non-specific reactions in serological assays *(Compton and Riley, 2001)*. Additional problems associated with serological assays should be considered when the MAP test is applied *(Parker et al., 1979; Kraft and Meyer, 1986; Besselsen et al., 2000)*. Furthermore, procedures for MAP testing of biological materials are not standardized. There are variations in the strain, age, gender and number of mice used, route of inoculation, volume of inoculum and the interval to permit seroconversion *(Homberger et al., 1991; Nicklas and Weiss, 2000; Bootz et al., 2003; Livingston et al., 2004)*. In addition, mice must be quarantined to prevent cross infection. Therefore, the MAP test is expensive and time-consuming.

Infant mouse bioassay

The infant mouse bioassay involves inoculation of suckling mice born to virus antibody-free mothers. Inoculation routes are intracranial, intranasal, oral or intraperitoneal. Mice are examined daily for morbidity or mortality for 14 days *(Homberger et al., 1991)*.

This method may reveal previously unknown viruses, as is the case with the MAP test. However, it requires confirmation of virus identity by further testing such as virus isolation, serological and histopathologic examinations. Inoculated mice must also be quarantined. Further, this method, while more rapid than the MAP test, takes longer than the PCR assay. These disadvantages make this method an obvious candidate for the replacement and reduction part of the 3R principle.

Gel-based PCR

The PCR is based on the amplification of nucleic acid sequences specific to a given virus. It can provide the desired amplification directly for DNA viruses or through use of reverse transcriptase PCR (RT-PCR) to detect RNA viruses after generation of complementary DNA (cDNA) *(Compton and Riley, 2001)*.

PCR is highly sensitive but is also susceptible to technical artefacts if not performed with extraordinary care including strict quality control of reagents, procedures and the laboratory environment. PCR can reveal viruses in biological material within hours or days, as opposed to the MAP test, which normally takes about five weeks. Additionally, PCR is more economical than the MAP test, despite the relatively high cost of reagents and equipment. Last, PCR reduces risks of exposure to zoonotic viruses such as LCMV *(Hinman et al., 1975)* and hantaviruses *(Lloyd and Jones, 1986)* since their infectivity is destroyed during nucleic acid extraction. As a major asset, PCR does not require the use of mice and therefore contributes to the 3R principle *(Russell and Burch, 1959)*.

There are, however, pitfalls associated with PCR; for example, polymerase inhibitors as reported for the detection of LDV *(Lipman et al., 2000a)*. This problem may be overcome by including spiked samples. Moreover, RNA is labile and deteriorates quickly if sample preparation is flawed. False-positive data resulting from nucleic acid contamination can be avoided by separation of pre- and post-amplification steps. In general, PCR results should be verified by sequencing, restriction analysis or hybridization.

Real-time/TaqMan/Fluorogenic nuclease

Real-time PCR is a quantitative variation of PCR based on the generation of a fluorescence signal during PCR amplification that corresponds to the quantity of DNA present in a specimen *(Henderson et al., 1999; Kendall et al., 2000)*. The increase in fluorescence is measured online and the data is transmitted to an attached computer in 'real' time. Real-time PCR eliminates post-PCR processing and carry-over contamination since it employs a closed-tube detection system. In addition, results are obtained faster than with the gel-based PCR.

Direct comparison of the sensitivity of methods for detection of murine viruses in biological materials

Data on the comparative sensitivities of the different methods from 1959 to date (Table 2) show that the MAP test was the most used method (n = 56), followed by PCR (n = 43), real-time PCR (n = 24), virus isolation in cell culture (n = 23) and infant mouse bioassay (n = 7). As expected, the comparisons with PCR assays are prominent more recently, coincident with its discovery and widening diagnostic applications since 1991 (*Homberger et al., 1991*). Comparisons were either between two (n = 48) or among three methods (n = 19), the majority comparing the MAP test with gel-based PCR (n = 37).

Sensitivity of virus isolation in cell culture

Virus isolation in cell culture was equally sensitive to the MAP test for detection of mouse polyoma virus *(Rowe et al., 1959)*, MMV *(de Souza and Smith, 1989; Mahabir et al., 2004)*, Reo3 *(de Souza and Smith, 1989)*, Sendai *(Parker and Reynolds, 1968)*, Ectromelia virus *(Wallace and Buller, 1985; Dick et al., 1996)* and LDV *(Nakai et al., 2000)* but was less sensitive than the MAP test for detection of Mad *(Hartley and Rowe, 1960)*, MHV *(de Souza and Smith, 1989; Mahabir et al., 2004)* and LCMV *(de Souza and Smith, 1989)*. Similar sensitivity was reported for virus isolation in cell culture and the infant mouse bioassay for detecting Mad *(Hartley and Rowe, 1960)* and K virus *(Greenlee et al., 1982)*.

Similar sensitivities using virus isolation in cell culture and gel-based PCR were reported for MMV *(Haag et al., 2000)*, Ectromelia virus *(Dick et al., 1996)* and Hantaan virus *(Xiao et al., 1991; Kariwa et al., 1995)*. Virus isolation in cell culture was less sensitive than the PCR for detecting MMV *(Garnick, 1998; Mahabir et al., 2004)*, LCMV *(Ciurea et al., 1999)* and MCMV *(Palmon et al., 2000)*. Interestingly, virus isolation on L929 cells was reported to be less sensitive than the gel-based PCR for detection of MMV but at least 10^3 times more sensitive for detecting MHV-A59 *(Mahabir et al., 2004)*.

Virus isolation in cell culture and real-time PCR showed similar sensitivities for MCMV detection *(Wheat et al., 2003)*. However, real-time PCR was more sensitive in detecting MCMV *(Vliegen et al., 2003)* and MHV *(Henderson et al., 1999)*. Using human newborn kidney cells (NB324K cells) to detect MMVp, the real-time PCR was even 10^3 times more sensitive *(Zhan et al., 2002)*.

Taken together, with the exception mentioned above, the sensitivity of virus isolation in cell culture was similar to or less than that of the MAP test, the infant mouse bioassay, gel-based PCR and real-time PCR.

Sensitivity of the MAP test

The MAP test showed similar sensitivity as the infant mouse bioassay for detecting LCMV *(Lewis and Clayton, 1971)*. It was less sensitive in detecting TMEV *(Lewis and Clayton, 1971)* and MTV *(Morse, 1990)* but detected more Mad *(Hartley and Rowe, 1960)* and 10 times more MHV *(Homberger et al., 1991)* and Reo3 *(Lewis and Clayton, 1971)* than the infant mouse bioassay. These reports indicate that the sensitivity of the MAP test and the infant mouse bioassay is virus-dependent.

While the MAP test and gel-based PCR detected similar amounts of MHV *(Homberger et al., 1991; Bootz et al., 2003; Livingston et al., 2004; Bauer et al., 2004)*, Reo3 *(Bootz et al., 2003; Livingston et al., 2004; Bauer et al., 2004),* TMEV *(Bootz et al., 2003)*, Ectromelia virus *(Lipman et al., 2000b)*,

Table 2. Comparative sensitivity of different methods used for detection of murine viruses in biological materials

Virus	Virus isolation in cell culture	MAP test	Infant mouse bioassay	Gel-based PCR	Real-time PCR
Mad	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [$10^{1.5}$]	(Bootz et al. 2003) [$10^{1.5}$]
	(Hartley and Rowe 1960)	(Hartley and Rowe 1960) [?]	(Hartley and Rowe 1960)	n. d.	n. d.
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004) [?]	n. d.
		(Bauer et al 2004)		(Bauer et al 2004) [10^{1}]	
MHV	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003)	(Bootz et al. 2003)
	(de Souza and Smith 1989)	(de Souza and Smith 1989) [10^{3}]	n. d.	n. d.	n. d.
	(Henderson et al. 1999)	n. d.	n. d.	n. d.	(Henderson et al. 1999) [10^{1}]
	n. d.	(Homberger et al. 1991) [10^{1}]	(Homberger et al. 1991)	(Homberger et al. 1991) [10^{1}]	n. d.
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004)	n. d.
	(Mahabir et al. 2004) [10^{3}]	(Mahabir et al. 2004) [$\geq 10^{4}$]	n. d.	(Mahabir et al. 2004)	n. d.
	n. d.	(Yamada et al. 1993)	n. d.	(Yamada et al. 1993) [3 x 10^{4}]	n. d.
		(Bauer et al 2004)		(Bauer et al 2004)	
K virus	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [10^{5}]	(Bootz et al. 2003) [10^{7}]
	(Greenlee et al. 1982)	n. d.	(Greenlee et al. 1982)	n. d.	n. d.
Rotavirus	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [10^{3}]	(Bootz et al. 2003) [10^{3}]
MPV	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004)	n. d.
	n. d.	(Redig and Besselsen 2001)	n. d.	n. d.	(Redig and Besselsen 2001) [$\geq 10^{2}$]
		(Bauer et al 2004)		(Bauer et al 2004)	
MMV	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [$10^{1.5}$)	(Bootz et al. 2003) [$10^{4.5}$]
	(de Souza and Smith 1989)	(de Souza and Smith 1989)	n. d.	n. d.	n. d.
	(Garnick 1998)	n. d.	n. d.	(Garnick 1998) [10^{2}]	n. d.
	(Haag et al. 2000)	n. d.	n. d.	(Haag et al. 2000)	n. d.
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004) [?]	n. d.
	(Mahabir et al. 2004)	(Mahabir et al. 2004)	n. d.	(Mahabir et al. 2004) [$\geq 10^{6}$]	n. d.
	n. d.	(Redig and Besselsen 2001)	n. d.	n. d.	(Redig and Besselsen 2001) [$\geq 10^{2}$]
	(Zhan et al. 2002)	n. d.	n. d.	n. d.	(Zhan et al. 2002) [10^{3}]
		(Bauer et al 2004)		(Bauer et al 2004) [10^{1}]	
PVM	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [10^{2}]	(Bootz et al. 2003) [10^{2}]
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004)	n. d.
		(Bauer et al 2004)		(Bauer et al 2004)	
Reo3	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003)	(Bootz et al. 2003) [10^{1}]
	(de Souza and Smith 1989)	(de Souza and Smith 1989)	n. d.	n. d.	n. d.
	n. d.	(Lewis and Clayton 1971) [10^{1}]	(Lewis and Clayton 1971)	n. d.	n. d.
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004)	n. d.
	n. d.	(Uchiyama and Besselsen 2003) [10^{1}]	n. d.	n. d.	(Uchiyama and Besselsen 2003)
		(Bauer et al 2004)		(Bauer et al 2004)	
Sendai virus	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [10^{2}]	(Bootz et al. 2003) [10^{3}]
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004) [?]	n. d.
	(Parker and Reynolds 1968)	(Parker and Reynolds 1968)	n. d.	n. d.	n. d.
	n. d.	(Wagner et al. 2003)	n. d.	n. d.	(Wagner et al. 2003)
		(Bauer et al 2004)		(Bauer et al 2004)	

Virus	Virus isolation in cell culture	MAP test	Infant mouse bioassay	Gel-based PCR	Real-time PCR
TMEV	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003)	(Bootz et al. 2003) [10^1]
	n. d.	(Lewis and Clayton 1971)	(Lewis and Clayton 1971) [$10^{2.2}$]	n. d.	n. d.
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004) [?]	n. d.
		(Bauer et al 2004)		(Bauer et al 2004) [10^1]	
Ectromelia virus	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [10^1]	(Bootz et al. 2003) [10^1]
	(Dick et al. 1996)	(Dick et al. 1996)	n. d.	(Dick et al. 1996)	n. d.
	n. d.	(Lipman et al. 2000b)	n. d.	(Lipman et al. 2000b)	n. d.
	(Wallace and Buller 1985)	(Wallace and Buller 1985)	n. d.	n. d.	n. d.
Hantaan virus	(Kariwa et al. 1995)	n. d.	n. d.	(Kariwa et al. 1995)	n. d.
	(Xiao et al. 1991)	n. d.	n. d.	(Xiao et al. 1991)	n. d.
LDV	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003) [10^4]	(Bootz et al. 2003) [10^4]
	n. d.	(Chen and Plagemann 1997) [10^1-10^2]	n. d.	(Chen and Plagemann 1997)	n. d.
	n. d.	(Li et al. 2000)	n. d.	(Li et al. 2000)	n. d.
	n. d.	(Lipman et al. 2000a)	n. d.	(Lipman et al. 2000a)	n. d.
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004) [?]	n. d.
	(Nakai et al. 2000)	(Nakai et al. 2000)	n. d.	n. d.	n. d.
	n. d.	(Riley et al. 1999)	n. d.	(Riley et al. 1999)	n. d.
	n. d.	(Wagner et al. 2004) [10^1]	n. d.	n. d.	(Wagner et al. 2004)
		(Bauer et al 2004)		(Bauer et al 2004)	
LCMV	n. d.	(Besselsen et al. 2003) [10^2]	n. d.	n. d.	(Besselsen et al. 2003)
	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003)	(Bootz et al. 2003)
	(Ciurea et al. 1999)	n.d.	n. d.	(Ciurea et al. 1999) [10^2]	n. d.
	(de Souza and Smith 1989)	(de Souza and Smith 1989)	n. d.	n. d.	n. d.
	n. d.	(Lewis and Clayton 1971)	(Lewis and Clayton 1971)	n. d.	n. d.
MCMV	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003)	(Bootz et al. 2003) [10^2]
	(Palmon et al. 2000)	n. d.	n. d.	(Palmon et al. 2000) [?]	n. d.
	(Vliegen et al. 2003)	n. d.	n. d.	n. d.	(Vliegen et al. 2003) [?]
	(Wheat et al. 2003)	n. d.	n. d.	n. d.	(Wheat et al. 2003)
Mouse Polyoma virus	n. d.	(Bootz et al. 2003)	n. d.	(Bootz et al. 2003)	(Bootz et al. 2003) [10^4]
	n. d.	(Carty et al. 2001)	n. d.	(Carty et al. 2001)	n. d.
	n. d.	(Livingston et al. 2004)	n. d.	(Livingston et al. 2004) [?]	n. d.
	n. d.	(Riley et al. 1999)	n. d.	(Riley et al. 1999) [?]	n. d.
	(Rowe et al. 1959)	(Rowe et al. 1959)	n. d.	n. d.	n. d.
		(Bauer et al 2004)		(Bauer et al 2004)	
MTV	n. d.	(Morse 1990)	(Morse 1990) [10^1]	n. d.	n. d.
No. of reports (n)	*23*	*56*	*7*	*43*	*24*

Values in brackets [ ] denote the sensitivity of a particular method in comparison to the least sensitive method for the same virus
Direct comparisons are found in the same row: □ = the most sensitive method, □ = the second most sensitive method, ■ = the least sensitive method, two methods with the same colour or □ = equal sensitivity, n. d. = not done, ? = sensitivity was compared but no values were given

LDV *(Riley et al., 1999; Li et al., 2000; Lipman et al., 2000a)*, LCMV *(Bootz et al., 2003),* MCMV *(Bootz et al., 2003)*, polyoma virus *(Bootz et al., 2003)*, MPV *(Livingston et al., 2004; Bauer et al., 2004)* and PVM *(Livingston et al., 2004; Bauer et al., 2004)*, the MAP test was less sensitive for detecting Mad *(Bootz et al., 2003; Livingston et al., 2004; Bauer et al., 2004)*, MHV *(Yamada et al. 1993)*, K virus *(Bootz et al., 2003)*, Rotavirus *(Bootz et al., 2003)*, MMV *(Bootz et al., 2003; Livingston et al., 2004; Mahabir et al., 2004; Bauer et al., 2004)*, PVM *(Bootz et al., 2003)*, Sendai *(Bootz et al., 2003; Livingston et al., 2004; Bauer et al., 2004)*, TMEV *(Livingston et al., 2004; Bauer et al., 2004)*, Ectromelia virus *(Bootz et al., 2003)*, LDV *(Riley et al., 1999; Bootz et al., 2003; Livingston et al., 2004; Bauer et al., 2004)* and polyoma virus *(Riley et al., 1999; Carty et al., 2001; Livingston et al., 2004; Bauer et al., 2004)*. In contrast, for MHV *(Mahabir et al., 2004)* and LDV *(Chen and Plagemann, 1997)*, the MAP test was reported to be more sensitive than the gel-based PCR.

The MAP test was equally sensitive to the real-time PCR in detecting MHV *(Bootz et al., 2003)*, LCMV *(Bootz et al., 2003)* and Sendai virus *(Wagner et al., 2003)* but at least 100 times less sensitive in detecting MMV and MPV *(Redig and Besselsen, 2001)*. In the latter case, the real-time PCR detected as little as 10 copies of target template. Similarly, the MAP test was less sensitive in detecting 12 from 14 of the viruses listed in Table 2 *(Bootz et al., 2003)*. In contrast, for detection of Reo3 *(Uchiyama and Besselsen, 2003)*, LCMV *(Besselsen et al., 2003)* and LDV *(Wagner et al., 2004)*, the MAP test was more sensitive than the real-time PCR.

Sensitivity of the infant mouse bioassay

For detection of MHV *(Homberger et al., 1991)*, the infant mouse bioassay was 10-fold less sensitive than the gel-based PCR. To our knowledge, no data are currently available on the direct comparison of this method with real-time PCR for viral diagnostic.

Sensitivity of the PCR

Current information shows increased application of the PCR for detecting murine viruses (Table 2). The most comprehensive report given to date compares the techniques of the MAP test, gel-based PCR and real-time PCR *(Bootz et al., 2003)* showing that the gel-based PCR was less sensitive than the real-time PCR for detecting K virus, MMV, Reo3, Sendai, TMEV, MCMV and polyoma virus whereas similar sensitivities were reported for Mad, MHV, Ectromelia virus, Rotavirus, PVM, LDV and LCMV.

Similarly, high sensitivities using only real-time PCR were recently reported for Mad, MCMV, MMV/MPV, Reo3, TMEV and LCMV *(Blank et al., 2004*). In fact for MMV/MPV, LDV, MHV, Hantaan and Seoul viruses, the detection limit of the real-time PCR was less than 10 genomic copies *(Blank et al., 2004)*. The different sensitivities in real-time PCR reported by the two groups are possibly due to differences in methodologies and read-outs.

Conclusion

Today, the number of mouse colonies that are health monitored on a regular basis is constantly increasing. The emerging data provide current knowledge of the microbiological status at a given time and, in turn, result in the availability of microbiologically highly defined mice. As a consequence, the risk of microbiological contamination of such mice or their derivatives can be kept to a minimum. This overview shows that the use of the PCR assay for screening biological materials for murine viruses is of increasing importance. However, recent work performed in our laboratory *(Mahabir et al., 2004)* showed that it is insufficient to use only one method for detection of viral agents in biological materials and our results imply that the most sensitive method should be assessed for each virus individually. This view is supported by some of the data presented in this overview. Given the limitations of the available diagnostic methods for quality assurance monitoring programmes, a combination of different diag-

nostic methods described here may prove helpful in detecting murine viruses in biological materials. As such, the information presented in this overview may aid in the decision-making process as to which method(s) could be adequately employed for obtaining reliable results when biological materials are screened for murine viruses.

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