

HyTest NEWS



Veterinary



Avian

1. Influenza A haemagglutinin H5

Avian influenza viruses occurring naturally among birds cause avian influenza infection. Usually “avian influenza virus” refers to influenza A viruses found mainly in birds, but infections with these viruses can occur also in humans. Avian influenza was first identified over 100 years ago during an outbreak in Italy. Since then, the disease has cropped up at irregular intervals in all world regions.

There are many different subtypes of type A influenza viruses and they differ because of changes in certain proteins on the surface of the influenza A virus (hemagglutinin [HA] and neuraminidase [NA] proteins). Many different combinations of HA and NA proteins are possible and each combination represents a different subtype. Of the 16 different hemagglutinin types only strains within the H5 and H7 subtypes cause highly pathogenic avian influenza, which is highly contagious and rapidly fatal in susceptible avian species. When highly pathogenic influenza H5 viruses cause outbreaks, the mortality rate among poultry is usually between 90 %- 100 %.

Highly pathogenic for mice avian influenza H5N1 strain was prepared as allantoic fluid of SPF chicken embryos. The virus was purified by ultracentrifugation. Inactivated virus vaccine was prepared by ether treatment and homogenization with complete Freund's Adjuvant. Balb/c mice were vaccinated by injection of 10 µl of the vaccine into each hind foot pat. The vaccination was repeated twice within one week. Spleen and lymph nodes were used for hybridoma production. Splenocytes and lymphocytes were fused with Sp 2/0 mouse myeloma by PEG-6000 and clones were grown in 96-well plates with selective HAT medium for 10 days. Screening of the MAb producing clones was performed by Hemagglutinin Inhibition (HI) test and by indirect ELISA. Positive clones were recloned using mouse peritoneal macrophages as a feeder. Selected 5 MAbs show high specificity for the hemagglutinin H5 in hemagglutinin inhibition (HI) test. All MAbs are suitable for indirect ELISA. For sandwich immunoassay we can recommend MAbs 19C11 or 11A9 for coating and MAbs 15A6 or 8D2 for detection. MAbs are working also in Dot blot application.

Ordering information:

Product	Cat.#	MAb	Isotype	Remarks
Anti-Influenza A haemagglutinin H5	3H5N	8D2	IgG2a	HIT, EIA, Dot blot
Anti-Influenza A haemagglutinin H5	3H5N	11A9	IgG2a	HIT, EIA, Dot blot
Anti-Influenza A haemagglutinin H5	3H5N	15A6	IgG2a	HIT, EIA, Dot blot
Anti-Influenza A haemagglutinin H5	3H5N	18D5	IgG2a	HIT, EIA, Dot blot
Anti-Influenza A haemagglutinin H5	3H5N	19C11	IgG2a	HIT, EIA, Dot blot
Anti-Influenza A haemagglutinin H5	3H5N	6C8	IgG1	HIT, EIA
Anti-Influenza A haemagglutinin H5	3H5N	7E6	IgG2a	HIT, EIA
Anti-Influenza A haemagglutinin H5	3H5N	1C7	IgG2a	HIT, EIA
Anti-Influenza A haemagglutinin H5	3H5N	6B4	IgG2a	HIT, EIA
Anti-Influenza A haemagglutinin H5	3H5N	9B3	IgG2a	HIT, EIA
Anti-Influenza A haemagglutinin H5	3H5N	2D1	IgG1	EIA
Anti-Influenza A haemagglutinin H5	3H5N	7D5	IgG2a	EIA



2. Marek disease virus (MD)

Marek disease (MD) is a highly contagious, lymphoproliferative disease of chickens. It is caused by the MD virus (MDV), an oncogenic avian herpesvirus. It is the most serious chronic concern to the poultry industry. Chickens are exposed at an early age to cell-free MDV through inhalation of contaminated dust. MDV-infected lymphocytes in the peripheral blood distribute the virus to other tissues. In susceptible chickens second round of cytolytic infection occurs after about 2 weeks. After 3 weeks the chronic inflamma-

tion of the peripheral nerves is often seen and changes in lymphoid cells may induce malignant transformation in this cell type. The disease is characterized by presence of T cell lymphoma as well as infiltration of nerves and organs by lymphocytes. Infected birds can suffer from asymmetric paralysis of one or more limbs, difficulty of breathing, dilation of the crop, depression and paralysis. Death occurs usually in a large number of birds (up to 80 percent).

Ordering information:

Product	Cat.#	MAb	Isotype	Remarks
Anti-Marek disease virus	3MD8	14C8	IgG3	EIA
Anti-Marek disease virus	3MD8	1G6	IgG2a	EIA
Anti-Marek disease virus	3MD8	5C2	IgM	EIA
Anti-Marek disease virus	3MD8	3G2	IgM	EIA
Anti-Marek disease virus	3MD8	3H9	IgG2b	EIA

3. Newcastle disease virus (NDV)

Newcastle disease (ND) is a highly contagious and sometimes fatal illness affecting many domestic and wild bird species. The causal agent, Newcastle disease virus (NDV), is a negative-sense single-stranded RNA virus. NDV affects the respiratory, nervous, and digestive systems. Clinical signs are extremely variable depending on the strain of virus, species and age of bird, concurrent disease, and pre-existing immunity. NDV is so virulent that many birds die without showing any clinical signs.

Transmission occurs by exposure to faecal and other excretions from infected birds, and through contact with contaminated food, water, equipment and clothing. Virus-bearing material can be picked up on shoes and clothing and carried from an infected flock to a healthy one. Exposure of humans to infected birds (for example in poultry processing plants) can cause mild conjunctivitis and influenza-like symptoms, but NDV otherwise poses no hazard to human health. MAbs are negative with parainfluenza type 3 and avian influenza hemagglutinins.

Ordering information:

Product	Cat.#	MAb	Isotype	Remarks
Anti-Newcastle disease virus	3ND5	9F7	IgG1	EIA, WB, HIT
Anti-Newcastle disease virus	3ND5	11F12	IgG2a	EIA, WB, HIT
Anti-Newcastle disease virus	3ND5	13H3	IgG2a	EIA, WB, HIT
Anti-Newcastle disease virus	3ND5	9C6	IgG2a	EIA, WB, HIT
Anti-Newcastle disease virus	3ND5	1C10	IgG2a	EIA, WB, HIT
Anti-Newcastle disease virus	3ND5	2H4	IgM	EIA, HIT
Anti-Newcastle disease virus	3ND5	8H2	IgG2a	EIA



Canine

1. Canine distemper virus (CDV)

Canine distemper is a contagious, incurable, often fatal, multisystemic viral disease that affects the respiratory, gastrointestinal, and central nervous systems. Distemper is caused by the canine distemper virus (CDV). It occurs among domestic dogs and many other carnivores, including raccoons, skunks, and foxes. CDV is fairly common in wildlife. Infected dogs shed the virus through bodily secretions and excretions, especially respiratory secretions. The primary mode of transmission is airborne viral particles that dogs breathe in. The development of a vaccine in the early 1960s led to a dramatic reduction in the number

of infected domestic dogs. It tends to occur now only as sporadic outbreaks.

For the production of MAb to CDV, splenocytes of the Balb/c mice recovered after acute infection by CDV envelope protein (Onderstepoort) were used. MAbs 8-1 and 5-4 against CDV could be used in ELISA with the native CDV and purified CDV, in immunofluorescence with infected Vero cells and in immunohistochemistry. These MAbs may be used for the detection of CDV in clinical samples (blood, saliva, tears, feces) of animals.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Canine distemper virus	3CD10	8-1	IgG2a	EIA (detection), PLA
Anti-Canine distemper virus	3CD10	5-4	IgG2	EIA (capture), PLA

2. Canine parvovirus (CPV)

Canine parvovirus (CPV) is a highly contagious and serious disease caused by a virus that attacks the gastrointestinal tract of puppies, dogs, and wild canids. Parvoviral infection must be considered as a possible diagnosis in any young dog with vomiting and/or diarrhea. Puppies and dogs usually become infected when they ingest virus that is passed in the feces of an infected dog. There is a 3-7 day incubation period before the puppy seems obviously ill. Canine parvovirus causes lethargy; loss of appetite; fever; vomiting; and severe, often bloody, diarrhea. Vomiting and diarrhea can cause rapid dehydration, and most deaths from parvovirus occur within 48 to 72 hours following

onset of clinical signs. Virus is shed for the first two weeks or less after infection in the stool of an infected dog.

MAbs 5G7 and 8H7 are directed against CPV. These MAb are working in ELISA with the native CPV and purified CPV, in immunodiffusion, hemagglutinin inhibition, immunohistochemistry and immunofluorescence. These MAbs may be used for the detection of CPV in clinical samples (foeces). MAbs 5G7 and 8H7 are cross-reacting with Mink enteritis virus and FPLV (*Feline panleucopenia*).

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Parvovirus, canine	3PV16	5G7	IgG2a	EIA (capture), WB, ID, HIT
Anti-Parvovirus, canine	3PV16	8H7	IgG2a	EIA (detection), WB, ID, HIT



3. Canine Adenovirus (CAV)

MAbs 8C4, 1E11 and 7C11 were produced by immunization with human adenovirus type 1 and boosting with canine hepatitis virus. These MAbs are working in ELISA with the native CAV and purified CAV

in immunodiffusion, immunohistochemistry and immunofluorescence. These MAbs may be used for the detection of Canine adenovirus infections in clinical samples (blood, nasal swabs) of animals.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Adenovirus hexon	3AV13	8C4	IgG2a	EIA (capture), ID, IHC
Anti-Adenovirus hexon	3AV13	7C11	IgG2a + IgM	EIA, ID, IHC
Anti-Adenovirus hexon	3AV13	1E11	IgG2a + IgM	EIA (detection), ID, IHC

4. Rabies virus

Rabies is a disease caused by a virus found in the saliva of infected animals and is transmitted to pets and humans by bites, or possibly by contamination of an open cut. It infects the central nervous system, causing encephalopathy and ultimately death. The time between exposure to the virus and the onset of symptoms can range from about two weeks to several months. The rabies virus can be found in animal saliva days before any obvious symptoms develop. However, all animals that have the virus will develop symptoms and eventually die of the disease. Most animals can be infected by the virus and can transmit the disease to humans. Infected bats, raccoons, foxes, skunks, dogs or cats provide the greatest risk to humans. For immunization we used vaccine strain of the rabies virus. Finally we got quite a number of

MAbs, which were characterized by the specificity to the glycoprotein (GP) and nucleoprotein (NP) of the rabies virus. MAbs 4G4 and 5B12 are specific to glycoprotein. MAb 1C5 against GP is of special interest. This MAb is very good in ELISA with the native RV and the purified GP and in immunofluorescence with intact infected BHK cells. Hybridoma cells, when inoculated into mice, protect them from RV infection and the MAb 1C5 demonstrates a therapeutic effect on the infected mice. MAb 1C5 has a virus-neutralizing activity in respect to the majority of standard RV and rabies-related viruses: CVS, Lagos bat, Mokola, Flury lep, Duvenhage and the field isolates. This antibody may be used for the detection of rabies virus and for creation of new vaccine anti-rabies preparations.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Rabies virus	3R7	1C5	IgG2a	EIA, IHC
Anti-Rabies virus	3R7	4G4	IgG2a	EIA
Anti-Rabies virus	3R7	5B12	IgG2a	EIA, WB



5. *Echinococcus granulosus*

Echinococcus granulosus, also called the Hydatid worm, is a cyclophyllid cestode that parasitizes the small intestine of canids as an adult, but which has important intermediate hosts such as livestock and humans, where it causes hydatid disease. In canids, *E. granulosus* causes a typical tapeworm infection. Adult

worms mature in the intestine of the dog (definitive host) and the eggs are released in the foeces. By an accidental ingestion in humans, oncospheres hatch in the duodenum, penetrate the intestine and are carried via the bloodstream to various organs. Hydatid cysts form in organs like liver, lungs and brain.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti- <i>Echinococcus granulosus</i>	3EG3	EHG	IgG1	EIA, WB

Bovine

1. *Brucella abortus* (Brucellosis)

Brucellosis is an infectious disease caused by the bacteria of the genus *Brucella*. Cows are the source of *Brucella abortus* but other species of *Brucella* can be contracted from other animals and can also cause brucellosis. The disease may be either subclinical, acute and subacute, relapsing or chronic. The incubation period may be weeks to months. It may be mild or an explosive, toxic illness. Symptoms are non-specific and few localizing physical signs develop. Diagnosis is usually from blood or bone marrow cultures, or a rise in anti-brucella antibodies of 4 fold or greater.

We used two antigens for anti-*B. abortus* MAb production: crude cell lysate and purified LPS. Respectively 2 panels of MAb were obtained: BA35, BA37, BA39 and Bx85, Bx87, Bx88. Selection of the optimal pair for *B. abortus* detection in sandwich-ELISA showed that MAb Bx85 is suitable for capture and MAb Bx88 works well and specifically as HRP-conjugate. Detection limit when using purified LPS as a standard is lower than 100 pg/ml, sensitivity is improved at sample boiling before the analysis.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti- <i>Brucella abortus</i>	3BR11	BA35	IgG2a	EIA, WB
Anti- <i>Brucella abortus</i>	3BR11	Bx85	IgG1	EIA (capture), LPS <i>Brucella abortus</i>
Anti- <i>Brucella abortus</i>	3BR11	Bx88	IgG1	EIA (detection), LPS <i>Brucella abortus</i>
Anti- <i>Brucella abortus</i>	3BR11	BrH1	IgG2a	EIA, WB
Anti- <i>Brucella abortus</i>	3BR11	BrF11	IgG1	EIA, WB
Anti- <i>Brucella abortus</i>	3BR11	BrG11	IgG2a	EIA, WB



2. Rotavirus

The incubation period for rotavirus disease is approximately 2 days. The disease is characterized by vomiting and watery diarrhea for 3 - 8 days, and fever and abdominal pain occur frequently. As with all viruses, some rotavirus infections cause few or no symptoms, especially in adults. Rotavirus spreads by fecal-oral transmission.

We used purified bovine rotavirus as an immunogen and MAb 3C10 is specific for mammalian group A rotaviruses. Antibody is cross-reacting with monkey rotavirus (SA-11), porcine rotavirus (PP) and with numerous human rotaviruses. Antibody is reacting in Western blotting with p42 major inner capsid antigen and it is working also in ELISA and immunohistochemistry.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Rotavirus Group Specific Antigen	3R10	3C10	IgG2a	P42 Antigen, EIA, IHC, WB

3. Bovine coronavirus

Bovine coronavirus infects neonatal calves and presents as an acute diarrhea. It frequently leads to death.

MAb 5A4 was produced by immunization with bovine coronavirus. MAb recognizes bovine coronavirus surface antigen (peplomer).

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Bovine corona virus	3BCV1	5A4	IgG1	EIA, HIT

4. Alpha-1 – Acid Glycoprotein (AGP)

The Alpha-1 – Acid Glycoprotein (AGP) level in 152 Holsteins from 1 year to 12 years is 283 µg/ml. There are no differences between male and female or between breeds. The upper limit of normal AGP in healthy bovine is 450 µg/ml. The serum value of AGP in a calf immediately following birth is 300 to 1750 µg/ml, but this drops to normal by the end of the third week following birth. Levels of AGP that re-

mains high longer than the 3-d week are indicative of a background health problem that will adversely affect growth and productivity. AGP is elevated in stressed cattle that are clinically normal. In particular the levels are elevated with hepatitis, pericarditis, arthritis, mastitis and pneumonia. With successful treatment or stress removal levels return to normal rapidly.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Alpha-1-Acid Glycoprotein, Bovine (Orosomuroid)	5AG1	GPB2	IgG1	EIA, WB
Alpha-1-Acid Glycoprotein, Bovine (Orosomuroid)	5AG1	GPB6	IgG2a	EIA, WB
Alpha-1-Acid Glycoprotein, Bovine (Orosomuroid)	5AG1	GPF7-2	IgG2b	EIA, WB
Alpha-1-Acid Glycoprotein, Bovine (Orosomuroid)	5AG1	GPC12	IgG2a	EIA, WB



5. Foot-and-mouth disease (FMDV)

MAbs against virulent serotype O1 of foot-and-mouth disease (FMDV) were screened in different serological reactions like ELISA and gel precipitation, in which both antibodies were active. Antibodies can be used to detect FMDV in the field isolates for differentiating type “O” from other types, for virus purification and for antibody screening in competitive ELISA, etc. MAbs against the non-structural protein (NSP) of foot-and-mouth disease can be used for example for antigen purification and for antibody screening in the compet-

itive ELISA, etc. Combination of an ELISA test, that detects antibodies directed against FMDV viral non-structural proteins (NSPs) and a liquid phase blocking competitive ELISA for the detection of antibodies against the viral structural proteins (SPs strain specific), can be used in analysis of field samples allowed for a clear differentiation between infected and uninfected animals, with high specificity and sensitivity, regardless of the animal’s vaccination status.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Foot-and-mouth disease	3FM2	2D2	IgG2a	virulent serotype O1, EIA, ID
Anti-Foot-and-mouth disease	3FM2	3G8	IgG2a	virulent serotype O1, EIA, ID
Anti-Foot-and-mouth disease	3FM2	1G2	IgG2a	non-structural proteins, EIA
Anti-Foot-and-mouth disease	3FM2	1H4	IgG2a	non-structural proteins, EIA

Equine

1. Burkholderia (*Pseudomonas mallei*) (Glanders)

Burkholderia (Pseudomonas) mallei is a gram-negative, non-sporing bacilli and it is the etiologic agent of glanders. Glanders is a highly contagious disease of solipeds and it is characterized by nodular lesions of the lungs and other organs, as well as ulcerative lesions of the skin and mucous membranes of the nasal cavity and respiratory passages. It is introduced into horse populations by diseased or latently infected animals. Ingestion of the pathogen, present in secretions from infected animals, constitutes the major

route of infection in glanders. Carnivores are susceptible to disease if they consume glandered meat. Humans also are susceptible to infection with glanders, which is an important occupational disease of veterinarians, farriers, and other animal workers. We have used cell extract of *Pseudomonas mallei* to develop a monoclonal antibody for detection of *Pseudomonas mallei*. MAb 3D11 MAb reacts with LPS of *Burkholderia mallei* in ELISA and Western blotting.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti- <i>Pseudomonas mallei</i> LPS	3PM15	3D11	IgG1	EIA, WB

Piscine

1. Infectious Salmon Anemia virus

Infectious salmon anemia virus (ISAV) is the causative agent of ISA, which is a highly infectious disease of farmed Atlantic salmon in the Northern hemisphere. This virus is a member of the family *Orthomyxoviridae*, genus *Isavirus*. We have used two synthetic peptides of Infectious Salmon Anemia Virus from putative

haemagglutinin sequence, conjugated to KLH to generate monoclonal antibodies suitable for EIA and WB. All MAb recognize peptides of putative haemagglutinin of Infectious Salmon Anemia Virus (8-23 a.a.r. for MAb 16C7 and 16F4 and 296-312 a.a.r. for MAb 18D8 and 18F9).

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Infectious Salmon Anemia Virus, Putative Haemagglutinin	3SA1	16C7	IgG1	a.a.r. 8-23, EIA
Anti-Infectious Salmon Anemia Virus, Putative Haemagglutinin	3SA1	16F4	IgG2a	a.a.r. 8-23, EIA
Anti-Infectious Salmon Anemia Virus, Putative Haemagglutinin	3SA1	18D8	IgG2b	a.a.r. 296-312, EIA
Anti-Infectious Salmon Anemia Virus, Putative Haemagglutinin	3SA1	18F9	IgG1	a.a.r. 296-312, EIA

Porcine

1. Transmissible Gastroenteritis (TGE) virus of Pigs

Transmissible gastroenteritis (TGE) is an acute highly contagious disease of pigs caused by virus from the Coronaviridae family. It is a common viral disease of the small intestine that causes vomiting and profuse diarrhea in pigs of all ages. There is high morbidity and mortality in piglets.

MAb 1E11 is specific to peplomer of TGE virus. MAb can be used in Immunometric detection of the TGE virus in stool and cell culture fluids. The same MAb can be used as capture and detection MAb. MAb can also be used in hemagglutinin inhibition.

Ordering information:

Product	Cat. #	MAb	Isotype	Remarks
Anti-Transmissible Gastroenteritis Pig Virus	3TG1	1E11	IgG1	EIA (capture, detection), HIT



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