医薬品 研究報告 調査報告書

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販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○日本(宮崎県)H5N1型高病原性トリインフルエンザ確定
2007年1月18日、農林水産省は、宮崎県の養鶏場で発生したトリインフルエンザは高病原性ウイルスによるものだったと明らかにした。同省は養鶏場で死亡した鶏から採取したウイルスのサンプルを検査して病原性が高いものであることを確認した。動物衛生研究所ではウイルスをH5N1型高病原性株と同定し、実験用鶏8羽にウイルスを接種したところ全て死亡したためこの結論に至った。H5N1型ウイルスの流行は、宮崎県清武町の谷口孵卵場黒坂農場で発生し、3つある鶏舎のうち1つで3500羽の鶏が死亡した。県の調査によると、他の養鶏場やペットの鳥などに異常は出ていない。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
宮崎県の養鶏場で発生したトリインフルエンザがH5N1型高病原性トリインフルエンザによるものだったと確定されたとの報告である。	日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。

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Archive Number 20070120.0260
Published Date 20-JAN-2007
Subject PRO/AH/EDR> Avian influenza (13): Japan H5N1, Viet Nam, Indonesia

AVIAN INFLUENZA (13): JAPAN H5N1, VIET NAM, INDONESIA
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail, a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1] Japan (Miyazaki) - HPAI H5N1 confirmed
[2] Viet Nam, Can Tho
[3] Indonesia

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[1] Japan (Miyazaki) - HPAI H5N1 confirmed
Date: Thu 18 Jan 2007
From: ProMED-mail <promed@promedmail.org>
Source: Kyodo News via Japan Times [edited]
<http://search.japantimes.co.jp/cgi-bin/nn20070119a4.html>

Bird flu virus in Miyazaki outbreak highly virulent

The avian influenza that broke out last week at a poultry farm in Miyazaki Prefecture involved a highly virulent virus, the Agriculture, Forestry and Fisheries Ministry said on Thursday [18 Jan 2007]. The ministry determined the degree of virulence through a laboratory examination of virus samples taken from chickens that died of bird flu on the Miyazaki farm.

The National Institute of Animal Health, which identified the virus as the highly pathogenic H5N1 strain on Tuesday [16 Jan 2007], drew the conclusion after 8 chickens inoculated with the sampled virus were dead by Thursday [18 Jan 2007], the ministry said. Based in Tsukuba, Ibaraki Prefecture, the institute will continue genetic analysis for the consideration of the ministry's panel of experts.

The H5N1 strain, a subtype of the influenza A virus circulating basically in birds, has spread mainly in Asia and has killed more than 160 people in 10 countries since 2003, according to the World Health Organization. Its latest outbreak in poultry in Japan -- the 5th bird-flu case here since 2004 -- occurred at Taniguchi Furanjo Kurosaka Farm in Kiyotake, Miyazaki Prefecture, causing the death of 3500 birds in one of the farm's 3 poultry houses, mostly last week.

In the town of Kiyotake, inspectors from the Miyazaki Prefectural Government's task force Thursday [18 Jan 2007] checked bird-keeping households and homes within a 10 km radius of the farm, after they finished examining the 11 poultry farms with more than 1000 chickens in the area Wednesday [17 Jan 2007].

Ten households with at least 20 birds, including chickens and bantams [small breeds of chickens], were selected for Thursday's [18 Jan 2007] on-site inspections to take blood and fluid samples from 5 birds per household, task force officials said, adding that results will be released within a few days.

More than 150 households are breeding a total of around 1600 avian species in the town, including parakeets and other small birds kept as pets, town officials said.

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[2] Viet Nam, Can Tho
Date: Fri 19 Jan 2007
From: Mary Marshall <tropical.forestry@btinternet.com>
Source: Reuters Alertnet [edited]
<http://www.alertnet.org/thenews/newsdesk/HAN277071.htm>

Bird flu found in ducks in Viet Nam city of Can Tho

Bird flu has killed ducks in the southern Mekong delta's largest city of Can Tho, as Viet Nam's animal health experts increase measures to contain the H5N1 virus, a government report said on Friday [19 Jan 2007]. The Animal Health Department said that tests showed H5N1 had killed ducklings in Can Tho 4 days after the virus was found in neighboring Soc Trang province. The report said the domesticated ducks had not been vaccinated against the virus.

The Agriculture Ministry has ordered a new round of poultry vaccinations and sent more animal health experts to try to stem the bird flu, which has struck 7 provinces and Can Tho city in the southern rice basket region in the past month as a harvest nears.

This week, the government ordered farmers to stop ducks from roaming in the Mekong delta. Animal health officials said H5N1 has killed nearly 19 000 poultry in the delta, mostly ducks. Not all ducks are killed by the virus, some being infected without showing symptoms. The birds still excrete the virus in their droppings as they paddle through muddy rice paddies looking for insects and leftover grain.

Viet Nam has had no human H5N1 cases since November 2005, but the virus that 1st hit the south east Asian country in late 2003 returned to the Mekong delta last month [December 2006]. Thursday's [18 Jan 2007] Lao Dong (Labour) newspaper said 23 people who ate ducks that died from unknown causes in Bac Lieu province have been put under surveillance.

The government is anxious to stop bird flu spreading ahead of the Tet Lunar New Year festival in mid-February [2007], when poultry is part of the traditional feast. Officials fear the disease could spread nationwide on the wings of migrating birds and through the movement of poultry, including the smuggling of chickens and ducks from neighboring countries.

Bird flu killed 42 of the 93 people infected in Viet Nam in 2003-2005. It has killed 161 people out of 267 infections globally since 2003, World Health Organization figures show.

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[For Can Tho, see province 18 in the interactive map at <http://www.angelfire.com/co/hongnam/vnmap.html>. - Mod.AS]

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[3] Indonesia
Date: Fri 19 Jan 2007
From: Mary Marshall <tropical.forestry@btinternet.com>
Source: Reuters Alertnet [edited]
<http://www.alertnet.org/thenews/newsdesk/JAK212987.htm>

Indonesian capital's bird cull gets mixed reaction

A campaign to rid the Indonesian capital of backyard fowl to fight bird flu got a mixed reaction from residents on Friday [19 Jan 2007], with some welcoming it, while others worried about losing a key source of income.

Jakarta governor Sutiyoso set fire to about 1600 chickens, ducks, and pigeons that had been slaughtered and dumped into a pit overnight in a densely populated neighborhood. Some residents responded with cheers of "Hurray!" at the launch of the campaign, although the culled birds were not known to have been infected by bird flu.

Sutiyoso told city residents on Wednesday [17 Jan 2007] that their backyard

fowl would be confiscated and destroyed if they failed to get rid of the birds by the end of the month. The move followed the deaths of 4 people in Jakarta and its surrounding areas since the start of the year [2007], which took the number of confirmed human deaths from the virus in Indonesia to 61, the highest in the world. "We support the government's program. It's better to do this than worrying about being infected," a resident said. But some residents were worried about the cost. Many people in Indonesia keep poultry to supplement their meager incomes. "The program is good but how can we eat eggs but not chicken?" she asked.

A trader at a nearby bird market said he had not sold a single bird since the governor announced the ban. He said that, even though traders like him could still sell birds, traders and owners would need to get a clean bill of health for birds from authorities. This could discourage buyers. "I hope officials can be present here to facilitate the certification," he said.

Health minister Siti Fadilah Supari on Thursday [18 Jan 2007] said the ban in Jakarta would be extended to 8 other provinces that had reported human infections of the H5N1 bird flu virus. More than a dozen people have been admitted to hospital with bird flu-like symptoms since the start of the year [2007], although many have been discharged or tested negative for the virus.

Indonesia faces an uphill task controlling the disease. Millions of backyard fowl live in close proximity to humans, and health education campaigns have often been patchy and rules difficult to enforce. Illustrating this, chickens roam freely just a few minutes walk from the capital's central business district.

Official calls for culling have also met stiff resistance in the past due to meager compensation and difficulties enforcing rules in the provinces. Officials said poultry owners would be paid compensation of 12 500 rupiah (USD 1.4) for each sick bird killed. A fully grown chicken costs about 35 000 rupiah [USD 4] in Jakarta. The governor said on Wednesday [17 Jan 2007] that there would be no payment for healthy birds.

[byline: By Heru Asprihanto]

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[No cases of avian influenza have been detected in Europe during the current winter season. It will be interesting to follow the results of this year's (2007) EU surveillance in wild birds. In 2006, infected wild birds were detected between February and May. Avian influenza is one of the main subjects for discussion during the due 25th Plenary meeting of EFSA'a (European Food Safety Authority) Animal Health & Animal Welfare panel, to be convened in Parma, Italy, 31 Jan - 1 Feb 2007. Among the topics: avian influenza - zoo birds vaccination; avian influenza vaccines; situation - migratory birds. - Mod.AS]

[see also:
Avian influenza (12): Hong Kong, Thailand, Japan, OIE 20070118.0237
Avian influenza, human (14): Indonesia, WHO 20070116.0204
Avian influenza (06): Viet Nam, OIE 20070113.0175
Avian influenza (02): Viet Nam 20070105.0050
Avian influenza: Viet Nam (Bac Lieu, Ca Mau, Hau Giang) 20070102.0015
Avian influenza (08): Japan (Miyazaki), H5 20070114.0184
2006

Avian influenza (225): Viet Nam (Bac Lieu, Ca Mau, Hau Giang) 20061231.3662]

.....arn/msp/sh

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2007年2月23日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①乾燥抗HBs人免疫グロブリン ②ポリエチレングリコール処理抗HBs人免疫グロブリン		研究報告の公表状況	Transfusion 2007; 47(3): 452-459	公表国 オーストリア	
販売名 （企業名）	①ヘブスブリン（ベネシス） ②静注用ヘブスブリンーIH（ベネシス）					

研究報告の概要

ヒトのH5N1ウイルス感染の増加が継続していることによって、これらウイルスがヒトの間で効率的に伝播する能力を獲得し、流行を起こす可能性がある。最近、より組織的なウイルスの伝播が、ヒトのH5N1ウイルス感染の間で報告され、その血液中に顕著なウイルス濃度が観察された。このことによって、輸血用血液製剤、ひいては血漿分画製剤の安全性についての懸念が高まった。
血漿分画製剤の安全性のマージンを確認するため、製造中のウイルス不活化工程について、ウイルスの不活化効果をH5N1インフルエンザウイルスの再集合体株を使って調査した。
その結果は、H5N1インフルエンザウイルスは、ヒトアルブミンの液状加熱、静注グロブリンのSD処理及び低pHインキュベーション、並びに第VIII因子インヒビターバイパス複合体製剤（FEIBA）の蒸気加熱工程において、HIV、BVDV、及びPRVのエンベロープウイルスと同様の挙動を示し、効率的に不活化された。
すなわち、H5N1インフルエンザウイルスの理論的伝播に対する血漿分画製剤の安全性マージンは、十分なものであった。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリンーIHの記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
ウイルス不活化実験の結果、H5N1インフルエンザウイルスは、HIV、BVDV、及びPRVのエンベロープウイルスと同様の挙動を示したとの報告である。 血漿分画製剤からの高病原性トリインフルエンザA（H5N1）ウイルス伝播の事例は報告されていない。また、万一原料血漿に高病原性トリインフルエンザウイルス(H5N1)が混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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H5N1 influenza virus and the safety of plasma products

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BACKGROUND: The ever-increasing number of human H5N1 influenza virus infections may enable these viruses to acquire the ability to spread effectively among humans and potentially to cause a pandemic. Recently, more systemic virus dissemination was reported during H5N1 virus infection of humans, resulting in significant virus concentrations also in the blood. The observation has raised concerns about the safety of labile blood products for transfusion and consequentially also for plasma derivatives. To confirm the safety margins of plasma products, dedicated virus inactivation processes used during their production were investigated for their effectiveness in inactivating this virus of recent concern.
STUDY DESIGN AND METHODS: Virus inactivation by steps commonly used during the manufacture of plasma derivatives, such as pasteurization for human albumin, solvent/detergent treatment for intravenous immunoglobulin (IVIG), vapor heating for factor VIII inhibitor bypassing activity, and incubation at low pH for IVIG, were investigated with a reassortant strain of H5N1 influenza virus.
RESULTS: The results show that H5N1 influenza behaves as expected for lipid-enveloped viruses; that is, the virus is effectively inactivated by all the commonly used virus inactivation procedures tested.
CONCLUSION: The safety margins of plasma derivatives against the theoretical transmission of H5N1 influenza virus are very substantial.

Influenza A viruses circulate in wild birds around the world and mostly coexist with their hosts. Since 1996, however, H5N1 viruses were recognized that had evolved into being more pathogenic for poultry and to occasionally also affect humans. To date, approximately 140 million domesticated birds have died from H5N1 virus infection or culling to prevent further virus spread,[1] and according to the WHO, of 233 humans reported infected 135 have died as of August 7, 2006.[2] This rather unusual pathogenicity of these H5N1 viruses for humans has been attributed to a resistance to the antiviral effects of interferons and tumor necrosis factor.[3] Also, H5N1 viruses that infect humans seem to have a broader tissue tropism when tested in ferrets[4] but also in the humans affected.[5,6] Once newly emerging influenza viruses acquire the ability to effectively spread among humans, these viruses can cause pandemics associated with significant morbidity and mortality as testified by several such episodes during the past century. Beyond those directly affected by virus infection, however, influenza viruses have generally been considered a relatively minor concern with respect to the safety of the blood supply, for example, based on the conclusions that viremia appears to be uncommon and that if viremia does occur it will be associated with fever and severe illness so that it is unlikely that these folks are going to want to donate.[7]

Careful review of available literature on the topic though, including less current and thus more difficult to access information, reveals several instances of reported influenza virus viremia in humans,[8-11] including cases where viremia has been described to occur in the absence of medical symptoms.[8,11] Reported several decades ago this information is still less comforting, although given our at present limited experience with H5N1 influenza, the relevance of the information to the current situation is not clear.

Recently, however, the presence—though not replication—of H5N1 virus was demonstrated in the spleen of an ultimately fatal human case, a finding that would support hematogenous spread of the virus.[5] Along those lines, the occurrence of influenza virus in the blood has been reported in two fatal cases of human H5N1 infection, at approximately 85,000[6] and 3,080[12] polymerase chain reaction–detectable copies per mL.

ABBREVIATIONS: BVDV = bovine viral diarrhea virus; FEIBA = factor VIII inhibitor bypassing activity; HA = human albumin; PRV = pseudorabies virus.

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TRANSFUSION 2007;47:452-459.

Based on currently available scientific evidence it would appear that one cannot be too certain about the level of concern that the H5N1 influenza viruses will, or not, pose to the safety of the blood supply, and by inference also the safety of plasma-derived medicines. For plasma-derived medicines though, a product class under particular scrutiny as it has in the past been involved in amplifying the spreading of emerging agents, additional reassurance can be derived from the dedicated inactivation procedures that are required to be incorporated into their manufacturing processes.[13] Although these processes have been validated for their effectiveness in inactivating other lipid-enveloped viruses, the available experimental evidence with orthomyxoviruses is very limited and to the author's best knowledge none for influenza H5N1 viruses. Validation studies with a range of physicochemically diverse viruses,[14] so-called model viruses, however, have generated evidence that these steps would provide very significant margins of safety also against H5N1 viruses for these products.

To provide additional reassurance, however, particularly for the patients who critically depend on plasma-derived products, but also regulatory authorities who need to justify the safety and thus presence of plasma derivatives on the market, studies aimed at confirming that influenza H5N1 viruses themselves would indeed behave as expected were performed for several major virus inactivation steps currently used during the manufacture of plasma derivatives.

MATERIALS AND METHODS

Viruses, cells, and infectivity assays

The human immunodeficiency virus (HIV), strain IIIB (National Institutes of Health [NIH], Bethesda, MD), was propagated on H9 cells (European Collection of Cell Cultures, Salisbury, UK; 85050301) and titrated on the Epstein-Barr virus transformed B lymphoblastoid cell line AA-2 (AIDS Research and Reference Reagent Program, NIAID, NIH, Bethesda, MD).

The bovine viral diarrhea virus (BVDV), strain Kentucky 22 (Biological Research Faculty & Facility, Ijamsville, MD), was propagated on MDBK cells (American Type Culture Collection [ATCC], Manassas, VA; CCL-22) and titrated on BT cells (ATCC CRL-1390). The pseudorabies virus (PRV), strain Kaplan (Federal Research Center for Virus Diseases of Animals, Tübingen, Germany), was propagated and titrated on Vero cells (European Collection of Cell Cultures 84113001).

The H5N1 influenza virus, strain NIBRG-14 (provided by J.M. Wood and J.S. Robertson from the National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK), was propagated on embryonated eggs and titrated on MDCK cells (ATCC CCL-34). NIBRG-14 H5N1 virus has been derived as a vaccine reference strain by reverse genetics, that is, the transfection of different influenza virus genes into one host cell that then produces the newly assembled virus (essentially as described in Nicolson et al.[15]). With respect to pathogenicity, the NIBRG-14 strain is very different from the currently circulating highly pathogenic H5N1 viruses, in that it is apathogenic. The virus particles, however, are physicochemically equivalent between these two viruses, as the surface glycoproteins hemagglutinin and neuraminidase are of H5N1 origin.

Virus-containing samples were titrated by $TCID_{50}$ assays with serial half-log dilutions of samples. Cells were incubated for 6 ± 1 days with samples before the cytopathic (BVDV, PRV, H5N1) or fusogenic (HIV) effect induced by the respective virus was evaluated. Virus concentrations were calculated according to the Poisson distribution and expressed as log ($TCID_{50}$) per mL.

Downscaled manufacturing processes for plasma derivatives

Downscaled versions of the manufacturing processes investigated were established, and their equivalence to the respective large scale processes was demonstrated by comparing critical process parameters and selected product parameters. Temperature as a critical process parameter for virus inactivation was monitored throughout all the processes investigated. The concentrations of virus-inactivating chemicals and the pH were measured, where relevant, to confirm equivalence with the manufacturing scale processes. Process intermediates were obtained from the manufacturing scale and used as starting materials, which were spiked 1:10 with virus stock suspensions. Immediately after spiking, samples were drawn and titrated to confirm the amount of virus added. Further samples were collected and titrated immediately at certain points during and at the end of the virus inactivation processes. Corresponding unspiked process intermediates were obtained from control runs and tested for their potential cytotoxicity for indicator cell lines and for their potential interference with the detection of low virus titers. Virus reduction factors for the manufacturing processes examined were calculated in accordance with Committee for Proprietary Medicinal Products (CPMP) guidance.[14]

Pasteurization of human albumin

During the pasteurization of final container of human albumin (HA), the liquid product is heat treated for 600 to 700 minutes at 60°C. Virus inactivation was investigated by heating 50 to 60 mL of the final product at 58°C, that is, 2°C below the temperature specified for manufacturing, for 600 minutes, which is the shortest possible treatment time. To provide further confidence in the robustness of the virus inactivation achieved, the heating process was evaluated with product of low and the highest HA concentrations, that is, 3.5 or 5 percent and 25 percent.

Vapor heating of anti-inhibitor coagulant complex, that is, factor VIII inhibitor bypassing activity

During the manufacture of factor (F)VIII inhibitor bypassing activity (FEIBA), a lyophilized intermediate of low residual moisture is heat treated in two phases, that is, first for a minimum of 510 minutes at 60°C, followed secondly by 60 minutes at 80°C. For the downscaled process, these heating steps were performed at the lower limit of these temperature specifications and for incubation times just below those specified for the manufacturing scale process. Also, separate runs were performed at the upper and lower limit of the residual moisture content as specified for manufacture to provide further assurance relative to the virus inactivation potential of the process. Product intermediate was spiked with virus, lyophilized, and then heat treated according to the procedure described above. FEIBA (clotting assay), FII activity (clotting assay), and FX activity (chromogenic assay), and protein concentration were determined for the downscale intermediate before and after the vapor-heating process, and the results were compared to the respective values for intermediates from the manufacturing scale to confirm equivalence of the different scale processes.

Solvent/detergent treatment of intravenous immunoglobulin (IVIG), that is, Gammagard Liquid/KIOVIG

During the intravenous immunoglobulin (IVIG), that is, Gammagard Liquid (Baxter Healthcare Corp., Westlake Village, CA)/KIOVIG (Baxter AG, Vienna, Austria), manufacturing process, intermediate product is incubated with the solvent/detergent (S/D) chemicals tri-*n*-butyl phosphate, octoxynol-9, and polysorbate 80, at target concentrations of 0.3, 1, and 0.3 percent, respectively, for at least 60 minutes at 18 to 25°C and pH 5.2. As earlier studies had shown virtually immediate virus inactivation under these conditions, only 50 and 5 percent of the nominal concentration of the virus-inactivating chemicals were used in experimental runs to provide a meaningful assessment of the kinetics of model and target virus inactivation. The equivalence of the downscale and manufacturing scale processes was further supported by monitoring of parameters like conductivity, pH, temperature, and measurement of the protein concentrations of the respective intermediates.

Low-pH treatment of IVIG (Gammagard Liquid/KIOVIG)

In the course of the manufacture of this IVIG preparation, the final product is incubated at a low pH of 4.4 to 4.9 and a temperature of 30 to 32°C for 21 to 22 days. To investigate virus inactivation by this procedure, the manufacturing scale intermediate was incubated at 29°C, that is, at the lower limit of the process specification, and at pH values of 4.4 and 4.9, that is, at the lower and the upper limit defined for the manufacturing scale process, and for up to 21 days, that is, the minimal incubation time for manufacture. To verify the equivalence of the downscale with the manufacturing scale process, pH and temperature, parameters critical for virus inactivation, were monitored throughout the incubation time.

RESULTS

Pasteurization of HA

Per European and US Pharmacopoeia requirements, the final HA product is subjected to pasteurization as a dedicated virus inactivation step. To create conditions least favorable to virus inactivation during the experimental investigations, the product was heat treated at only 58°C instead of at the 60°C process temperature specification.

In duplicate runs of 600 minutes' duration, with either low- (3.5 or 5%) or high-concentration (25%) HA intermediates, all the HIV, BVDV, and PRV that could be spiked into the product at the lower/upper limit of the specified protein concentration were completely inactivated after 30/30, 120/120, and 30/30 minutes, respectively, of the 600-minute heating process. Also, the H5N1 influenza virus was completely inactivated after 30/30 minutes of heating within material (Table 1). The reduction factors after the full 600-minute heating period were greater than 8.0/greater than 7.5 for HIV, greater than 6.4/greater than 6.2 for BVDV, greater than 7.7/greater than 7.2 for PRV, and greater than 7.6/greater than 7.0 for H5N1 influenza virus.

Vapor heating of FEIBA

Intermediate FEIBA was subjected to vapor heating in a downscaled process at temperatures and incubation times just below the specifications for these parameters during the manufacturing scale processes, in runs performed at residual moisture levels of 7 and 8 percent, that is, the lower and upper limit for manufacturing. Nevertheless, the combination of lyophilization and subsequent vapor heating inactivated all the model viruses as well as the H5N1 influenza virus to below the limit of detection during the two-phase heat treatment (Table 2), resulting in virus reduction factors of greater than 5.9/greater than 5.8 for HIV, greater than 5.6/greater than 5.6 for BVDV, greater than 6.7/greater than 6.6 for PRV, and greater than 5.3/greater than 5.1 for H5N1. Virus inactivation by the two-phase vapor-heating process was reconfirmed as being insensitive to slight variations in residual moisture content of the intermediates, in that there was no significant difference between virus inactivation at the upper

TABLE 1. Virus inactivation during pasteurization of HA*

	HIV		BVDV		PRV		H5N1	
HA concentration (%):	5	25	5	25	5	25	3.5	25
Virus stock suspension	8.0	8.0	7.1	7.1	7.4	7.4	7.3	7.3
Spiked process intermediate	6.9	7.0	6.0	5.8	6.7	6.6	6.6	6.5
Heating at								
58°C for 0-1 min	5.4	4.8	4.5	4.2	1.6	0.9	2.9	3.0
58°C for 30 min	<0.1	<0.6	1.8	0.6	<0.1	<0.6	<0.1	<0.6
58°C for 60 min	<0.1	<0.6	0.6	0.6	<0.1	<0.6	<0.1	<0.6
58°C for 120 min	<0.1	<0.6	<0.6	<0.6	<0.1	<0.6	<0.1	<0.6
58°C for 360 min	<0.1	<0.6	<0.6	<0.6	<0.1	<0.6	<0.1	<0.6
58°C for 600 min	<0.1	<0.6	<0.6	<0.6	<0.1	<0.6	<0.1	<0.6
600 min (bulk titration)	<(–0.8)	<(–0.3)	<(–0.3)	<(–0.3)	<(–0.8)	<(–0.3)	<(–0.8)	<(–0.3)
Reduction factor (log)†	>8.0	>7.5	>6.4	>6.2	>7.7	>7.2	>7.6	>7.0

* Results are reported as $TCID_{50}$ per mL. Negative log values for virus titers can be obtained when volumes greater than 1 mL are used for virus titration, for example, 1 infectious unit in 10 mL equals 0.1 unit per mL; the log of 0.1 is (–1). The same is true for the detection limit when no infectious virus is detected.

† Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).

TABLE 2. Virus inactivation during freeze-drying followed by vapor heating of FEIBA*

	HIV		BVDV		PRV		H5N1	
Residual moisture (%):	7.0	8.0	7.0	8.0	7.0	8.0	7.0	8.0
Virus stock suspension	7.4	7.2	7.0	6.7	8.0	7.7	6.3	6.5
Spiked process intermediate	6.2	6.1	6.0	5.5	7.1	7.0	5.3	5.0
Spiked and lyophilized intermediate	4.6	4.8	3.2	3.5	4.8	4.6	4.4	4.4
Heated to 59.0°C	3.5	3.2	2.7	2.6	1.1	1.4	4.2	4.2
Heated at								
59.5 ± 0.5°C for 180 min	1.9	2.0	1.1	0.9	1.8	<1.1	2.9	2.7
59.5 ± 0.5°C for 360 min	1.7	1.8	<1.1	<0.6	<1.1	<1.1	2.0	<0.6
59.5 ± 0.5°C for 505 min	1.9	1.4	<1.1	<0.6	<1.1	<1.1	<0.6	<0.6
Heated to 79.0°C	0.6	0.6	<1.1	<0.6	<1.1	<1.1	<0.6	<0.6
Heated at								
79.5 ± 0.5°C 30 min	<0.6	<0.6	<1.1	<0.6	<1.1	<1.1	<0.6	<0.6
79.5 ± 0.5°C 55 min	<0.6	<0.6	<1.1	<0.6	<1.1	<1.1	<0.6	<0.6
Reduction factor (log)†	>5.9	>5.8	>5.6	>5.6	>6.7	>6.6	>5.3	>5.1

* Results are reported as $TCID_{50}$ per mL.

† Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).

and the lower limits for the moisture content specified for the manufacturing procedure.

S/D treatment of Gammagard Liquid/KIOVIG

Investigating the S/D treatment of IVIG intermediates S/D chemicals were used at significantly reduced concentrations compared to those specified for the manufacturing scale process. By use of only half of the nominal concentrations of the S/D chemicals, that is, the minimum concentration allowable for manufacturing, BVDV, PRV, and H5N1 influenza were inactivated to below the limit of detection already after 2 minutes of the 60-minute treatment and HIV after 10 minutes. In an even more drastic experimental setting, only 5 percent of the S/D chemicals were added. Even these 20-fold lower concentrations compared to manufacturing resulted in a complete inactivation of HIV, BVDV, and H5N1 influenza, for BVDV and H5N1 already after 2 minutes of the incubation period. PRV was inactivated by 2.8 log. Because partial H5N1 inactivation occurred already after spiking the virus into the test article at pH 5.2, that is, before addition of the S/D chemicals, an additional experiment was performed with process intermediate rebuffered to neutral pH (7.0-7.4). As can be seen, the presence of 5 percent of the S/D chemicals compared to manufacturing still inactivated 1.9 log H5N1 influenza virus (Table 3).

Low-pH treatment of Gammagard Liquid/KIOVIG

When investigating the low pH treatment of the IVIG intermediate, HIV, BVDV, and PRV were completely inactivated by the low-pH treatment procedure, at pH values at both the upper and the lower limit of the manufacturing process specifications. The incubation times and temperatures applied in the downscale investigation were again those least favorable for virus inactivation, that is, the lowest possible temperatures and shortest possible incubation times (Table 4). The H5N1 virus, however, was inactivated instantaneously, as expected from the known

TABLE 3. Virus inactivation during S/D treatment of Gammagard Liquid/KIOVIG*

	HIV		BVDV		PRV		H5N1		
SD chemicals (% of specified):	50	5	50	5	50	5	5	50	5
pH value	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	7.0-7.4†
Virus stock suspension	7.9‡	7.9	8.3‡	8.1	8.0‡	7.7	7.2	7.4	7.3
Spiked process intermediate	7.6‡	7.6	8.6‡	8.2	7.8‡	8.4	6.3‖	3.1‖	7.1
2 min§	4.5‡	5.0	<3.7‡	<3.7	<4.0‡	7.0	<5.7	<3.7	6.2
10 min§	<4.5‡	4.9	<3.7‡	<3.7	<4.0‡	6.4	<5.2	<3.7	5.7
20 min§	<4.5‡	4.5	<3.7‡	<3.7	<4.0‡	6.0	<5.2	<3.7	5.7
30 min§	<4.5‡	3.7	<3.7‡	<3.7	<4.0‡	6.1	<4.7	<3.7	5.9
60 min§	<4.5‡	<3.7	<3.7‡	<3.7	<4.0‡	5.7	<4.7	<3.7	5.2
30 min (bulk titration)§	<3.6‡	ND	<2.8‡	<2.8	<3.1‡	ND	<3.8	<2.8	ND
60 min (bulk titration)§	<3.6‡	ND	<2.8‡	<2.8	<3.1‡	ND	<3.8	<2.8	ND
Reduction factor (log)	>4.5‡¶	>3.9	>6.2¶	>5.8¶	>5.1‡¶	2.8	>3.7¶	>4.7¶	1.9

* Results are given as $TCID_{50}$. ND = not determined
† Because H5N1 is inactivated at pH 5.2, an additional virus-spiked run was performed with intermediate rebuffered to pH 7.0 to 7.4.
‡ Mean of two runs performed with intermediate at the low or high end of the protein concentration specification.
§ Sample pretreatment, that is, samples containing S/D reagent were diluted 1:100 with 2 to 8°C cold cell culture medium prior to titration, was taken into account.
‖ The titer of the "spiked process intermediate" sample from the pH 7.0 to 7.4 run was used for calculation of the reduction factor.
¶ Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).

TABLE 4. Virus inactivation during low-pH incubation of Gammagard Liquid/KIOVIG*

	HIV		BVDV		PRV		H5N1	
pH:	4.4	4.9	4.4	4.9	4.4	4.9	4.4	4.9
Virus stock suspension	6.7	6.7†	6.8	6.7†	7.4	7.4†	6.9	6.9
Spiked process intermediate	5.5	5.5†	5.7	5.8†	6.4	6.3†	<1.1	<1.1
0 days (after reaching temperature)‡	4.9	5.4†	5.6	5.6†	5.5	6.2†	<1.6	<1.6
1 day‡	ND	ND	ND	ND	0.6	1.0†	<0.6	<0.6
2 days‡	<0.6	<0.9†	ND	ND	<1.1	<0.9†	ND	ND
4 days‡	<1.1	<0.9†	3.4	4.4†	<1.1	<0.9†	<0.6	<0.6
7 days‡	<1.1	<0.9†	3.1	2.9†	<1.1	<0.9†	<0.6	<0.6
10 days‡	<1.1	<0.9†	ND	ND	<1.1	<1.1†	<0.6	<0.6
14 days‡	<1.6	<1.1†	<1.6	1.1†	<1.6	<1.1†	<1.1	<1.1
20 days‡	<1.1	<1.1†	<1.6	<1.1†	<1.6	<1.4†	<1.1	<1.1
14 days (bulk titration)‡	<0.7	<0.2†	<0.7	NA/<0.7†§	<0.7	<0.2†	<0.2	<0.2
20 days (bulk titration)‡	<0.2	<0.2†	<0.7	<0.2†	<0.7	<0.5†	<0.2	<0.2
Reduction factor (log)‖	>5.7	>5.8†	>5.4	>5.8†	>6.4	>6.6†	>6.3¶	>6.3¶

* Results are given as $TCID_{50}$ per mL. ND = not determined; NA = not applicable.
† Mean of two runs.
‡ Sample pretreatment, that is, 1:3.16 dilution with 2 to 8°C cold cell culture medium before titration, was taken into account.
§ For one of the two runs, no titer could be calculated for the bulk titration due to the presence of positive wells.
‖ Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).
¶ Titer of "virus stock suspension" was used for calculation of the reduction factor, because complete H5N1 virus inactivation occurred already after spiking.

pH-dependent activation of the influenza virus hemagglutinin at pH 5.2 or lower.[16]

DISCUSSION

During the licensing process the safety margins of plasma-derived medicinal products with respect to potential virus transmissions are validated by generating and/or assessing experimental information on the behavior of a range of physicochemically diverse viruses, that is, model and target viruses, in downscaled models of the respective manufacturing processes. When facing emerging viruses, this information, which has inherently been designed to address different concerns, still forms the basis of predictions relative to the behavior of these other less well-known viruses. And while there is a notion that the plentiful information generated with a wide variety of viruses does still, at least for the more widely used inactivation procedures, allow for reasonably solid extrapolations towards which viruses should be more susceptible or resistant to these procedures, any newly emerging virus challenges these assumptions in a rather discomforting way.

With respect to influenza viruses, orthomyxoviruses have not been widely used as model viruses, and thus specifically for these viruses the available amount of information is limited, at best. For H5N1 itself, the available amount of information has been none. The NIBRG-14 virus used for the current investigation is physicochemi-