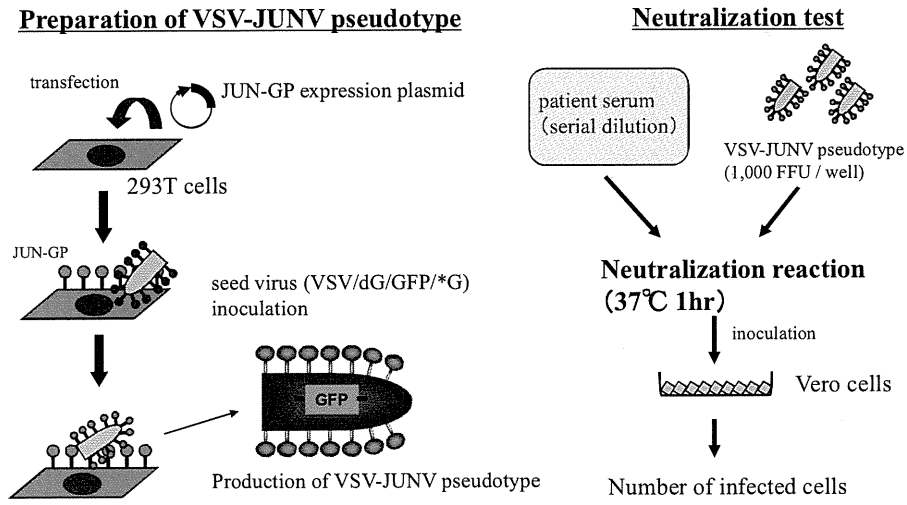
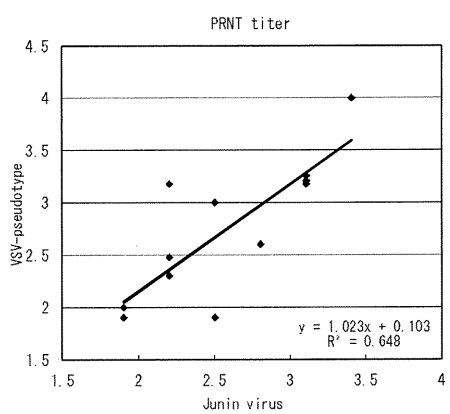
VSVシュードタイプを用いた安全で迅速な代替え中和抗体測定法の例



VSVシュードタイプを用いた代替え中和抗体測定法による南米出血熱（アルゼンチン出血熱）患者からの抗体検出

Sample	authentic	alternative
Neg control	20	<80
#77342	5	100
#77342	160	200
#77342	640	400
#77342	1280	1500
#77216	320	<80
#77216	160	300
#78401	160	1500
#78401	2560	10000
#51172	80	<80
#23139	160	200
#76482	1280	1600
#34600	1280	1800
#76916	320	1000
#34657	10000	2000
#52718	40000	4000



Comparison of authentic NT and alternative NT (Scatter diagram)

診断法の評価

- 海外のBSL4施設保有研究機関との共同研究などで評価
- 世界健康安全保障グループラボラトリーネットワークや国際高度安全実験室ネットワークのEQA参加
- 海外の流行地での調査・共同研究

Smallpox Diagnostics: Global Preparedness

Stephanie L. Lee[1], Antonio Di Caro[2], Anne Laure Favier[3], Allen R. Grolla[4], Sandra Lacote[5], Shigeru Morikawa[6], Andreas Nitsche[7], Hiram Olivera[8], Pia Zimmermann[9], Inger K. Damon[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, USA; [2]National Institute for Infectious Diseases, Lazzaro Spallanzani, Rome Italy; [3]Ministry of Defense, Medical Corps, Research Center CRRSA Emile Pardé, Grenoble, France; [4]Public Health Agency of Canada, Winnipeg, Canada; [5]Jean Mérieux BSL4/INSERM, Lyon, France; [6]National Institute of Infectious Diseases, Tokyo, Japan; [7]Robert Koch-Institut, Berlin, Germany; [8]National Institute for Epidemiological Diagnosis and Reference, Mexico City, Mexico; [9]Bundeswehr Institute of Microbiology, Munich, Germany

✧ **GHSAG Laboratory Network (GHSAGLN)** is a network of high level laboratories to co-ordinate, standardize and validate diagnostic capabilities and contribute to global health surveillance and response to disease outbreak (3). GHSAGLN also addresses challenges related to transporting diagnostic specimens and reference materials across international borders (4).

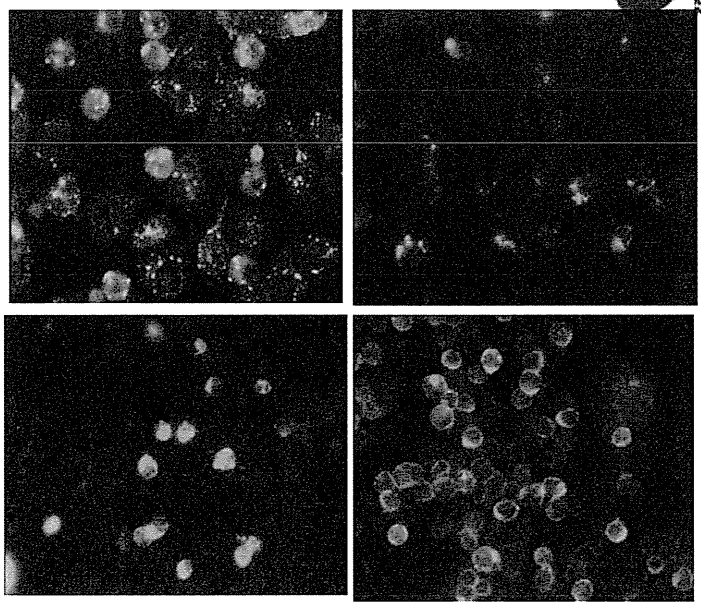
Summary of Results

✧ All participants correctly identified the variola strains - most detecting at the concentration level of 1fg/μl

✧ 3 out 6 countries identified an orthopoxvirus in the raccoonpox sample (North American orthopoxvirus)

✧ One country had a false positive result as variola in one of the negative controls and another country had an indeterminate result with the same control

IF signal of recombinant NP Ags



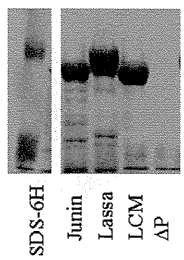
例） Sensitivity and specificity of IF method using EBO-rNP expressing HeLa cells

	IF positive	Negative
EBO-patient’s sera	14	0
Non-EBO-patient’s sera	1	51

Sensitivity; 100%
Specificity; 98%

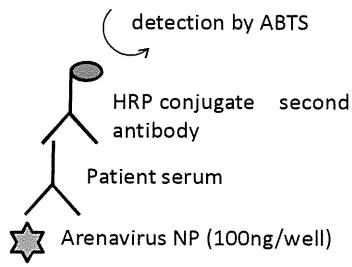
IgG-ELISA using recombinant arenavirus-NP

Purification of arenavirus NP



Expression and purification of Lassa, Junin, and other arenavirus NP by recombinant baculoviruses

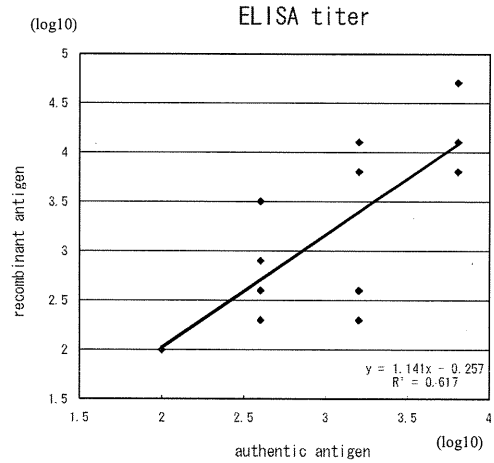
IgG-ELISA



例）Comparison of IgG-ELISA using authentic Junin Ags and rec NP

Sample	Viral Ag	rAg
Neg control	Neg	<100
#77342	Neg	<100
#77342	400	200
#77342	400	400
#77342	1600	12800
#77216	1600	400
#77216	400	3200
#78401	6400	12800
#78401	6400	51200
#51172	400	800
#23139	Neg	<100
#76482	6400	6400
#34600	1600	200
#76916	1600	6400
#34657	ND	400
#52718	ND	1600

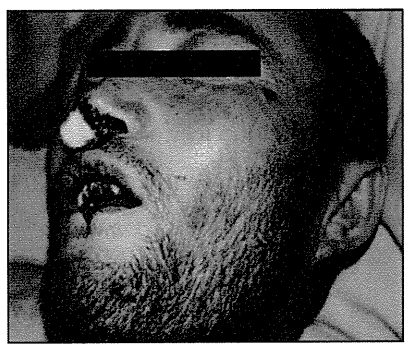
ELISA titer

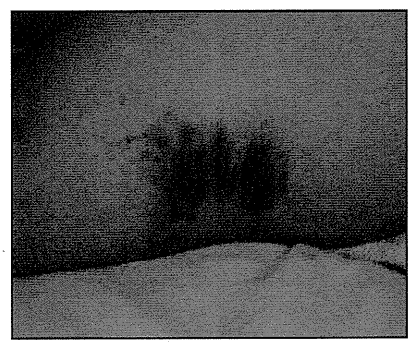


Comparison of rAG-based IgG- ELISA and Authentic IgG-ELISA (scatter diagram)

感染研のウイルス性出血熱診断法の適用例

CCHF patient in China in 2001

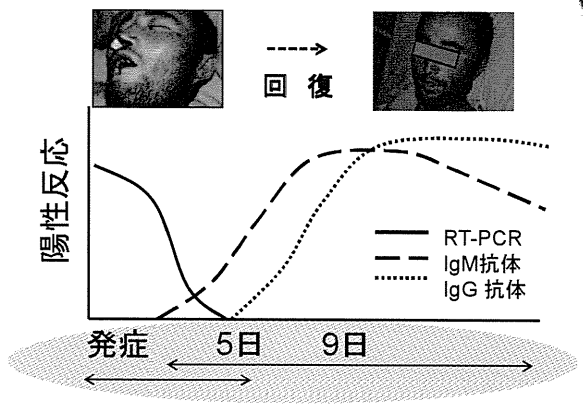




Laboratory test for the CCHF-patient 2001

Days from onset (Days)		1	5	9
RT-PCR (nested)		+	-	-
Ag-capture ELISA (OD_{405})		0.38	0.01	0.00
IgG-ELISA (OD_{405})	(1:100)	0.08	0.94	1.88
	(1:400)	0.03	0.49	0.97
IgM-capture ELISA (OD_{405})	(1:100)	0.00	2.69	2.71
	(1:400)	0.02	2.67	2.71

クリミア・コンゴ出血熱の場合



ウイルスの検出
- ウイルス分離
- RT-PCR
- 抗原検出ELISA

抗体の検出（血清診断）
- IgG抗体の上昇の確認（急性期と回復期）
- IgM抗体の検出

診断法のまとめ

感染症		発生	ウイルス	検査法 ELISA	IF	NT	Ag detection	PCR	LAMP
南米出血熱	アルゼンチン出血熱	Argentine	Junin	NP	NP / GP	VSV-pseudo	NP	(RT-PCR)	
	ボリビア出血熱	Bolivia	Machupo	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
	ベネズエラ出血熱	Venezuela	Guanarito	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
	ブラジル出血熱	Brazil	Sabia	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
	未定	Bolivia	Chapare	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
ラッサ熱		西アフリカ	Lassa	NP	NP / GP	VSV-pseudo	NP	RT-PCR	
未定		Zambia	Lujo	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
エボラ出血熱		DRC周辺	Zaire ebola	NP / GP	NP / GP	VSV-pseudo	NP	RT-PCR	
		Sudan	Sudan ebola	NP / GP	NP		NP	RT-PCR	
		Uganda	Bundibugyo ebola	(NP)	(NP)		(NP)	(RT-PCR)	
		Côte d'Ivoire	Cote d'Ivoire ebola	NP / GP	NP		NP	RT-PCR	
		Philippines	Reston ebola	NP / GP	NP / GP	VSV-pseudo	NP	RT-PCR	
マールブルグ病		Angola, Uganda, Keniya, DRC	Lake Victoria Marburg	NP	NP	VSV-pseudo	NP	RT-PCR	LAMP
ニパウイルス感染症		Malasia, Pakistan	Nipah	N/P/M/G		(VSV-pseudo)		RT-PCR	
ハンタウイルス肺症候群		北米	Sin Nombre	NP		dNP-ELISA		RT-PCR	
		南米	Andes	NP		dNP-ELISA		RT-PCR	
リフトバレー熱		アフリカ、中東	Rift valley fever	NP, virus	virus	VSV-pseudo, virus	NP	(RT-PCR)	
チクングニア熱		アフリカ、アジア	Chikungunya	virus (IgM)		virus		RT-PCR	
新興ウイルス感染症		?	?	遺伝子組換えの大臣確認実験申請では対応が間に合わない				CoCoMo primer RDV	

重篤な新興ウイルス感染症

最近のウイルス性出血熱等

- 新型・新種のウイルスによるウイルス性出血熱等
  - 新種の旧世界アレナウイルスによるウイルス性出血熱（ザンビア・南ア2008 / Lujo virus ）
  - 新種の新世界アレナウイルスによる南米出血熱（ボリビア2007、Chapare virus）
  - 新世界アレナウイルスの異なるcladeのウイルス間でのS-segment RNA内のNP遺伝子末端での組換えによる新型アレナウイルスの発生(カリフォルニア 1999/2000 / Whitewater Arroyo virus)
  - 新型（新種）のエボラウイルスの出現（ウガンダ2007-8、Bundibugyo ebolavirus)
  - Severe fever with thrombocytopenia syndrome (SFTS) (遼寧省、山東省、江蘇省、安徽省、湖北省、河南省2008)
- 輸入感染症としてのウイルス性出血熱の発生
  - マールブルグ熱のオランダ、米国での初めての患者発生
  - ラッサ熱患者の英国での発生
  - 想定されない動物からの危険なウイルスの検出
  - 輸入齧歯類からのサル痘患者発生（米国2003、約70名）
- 動物からのウイルス検出
  - ブタのエボラウイルス感染
  - オオコウモリからヘニパウイルスが検出（アジア諸国やアフリカ）
  - サルのジステンパーウイルス感染症（中国、日本2008）

Emergigng arenavirus HF in Zambia and South Africa in 2008

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Special Pathogens Branch

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Outbreak Postings

2008: Hemorrhagic fever due to novel Old World arenavirus, Zambia and South Africa ▲ to top

On October 2, CDC-Zambia notified CDC's Special Pathogens Branch about a cluster of 2 cases of a fatal febrile illness suspected to be a viral hemorrhagic fever, with probable person-to-person transmission. Both patients were medevaced from Zambia to South Africa and died there. During hospitalization, further transmission occurred in three other hospital workers, two of whom also subsequently died. Preliminary results indicate that the causative agent is a novel Old World arenavirus distinct from other arenaviruses such as Lassa and LCM. CDC's Special Pathogens Branch and Infectious Diseases Pathology Branch have been working closely with colleagues in CDC-Zambia, the Special Pathogens Unit, National Institute of Communicable Diseases (NICD) in South Africa, and CDC-South Africa as well as the respective National Ministries of Health to provide laboratory and epidemiologic support.

- WHO information about the cases in Zambia and South Africa
- National Institute for Communicable Diseases, South Africa information about the cases in Zambia and South Africa

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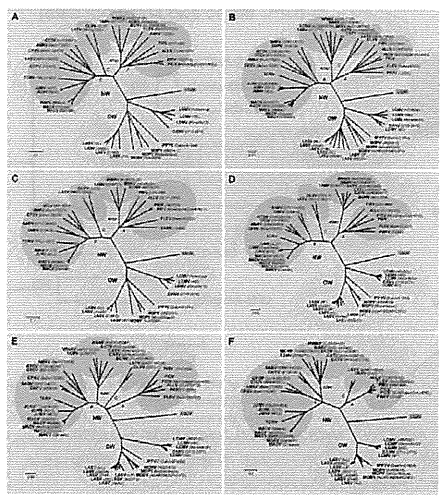
Genetic Detection and Characterization of Lujo Virus, a New Hemorrhagic Fever–Associated Arenavirus from Southern Africa



L (nuc) S (nuc)

L (aa) NP (aa)

G2(aa) G1(aa)

新種の旧世界アレナウイルスによるウイルス性出血熱の発生（ザンビア・南ア 2008 / Lujo virus）

旧世界アレナウイルスのいずれともかなり異なり、特にエンベロープ蛋白は旧世界と新世界アレナの中間に位置する。

A new arenavirus isolated from South American HF patient in Bolivia in 2008

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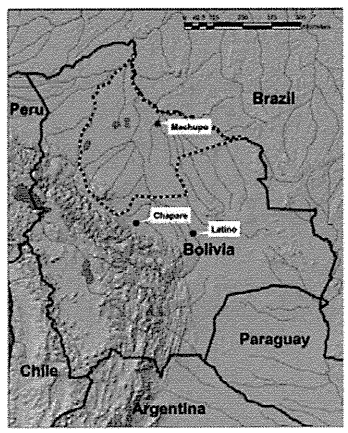
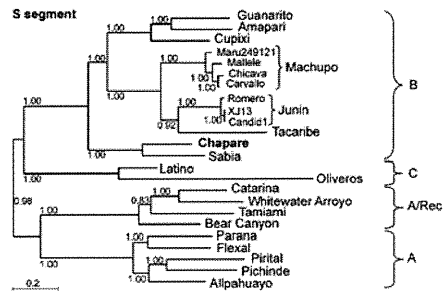
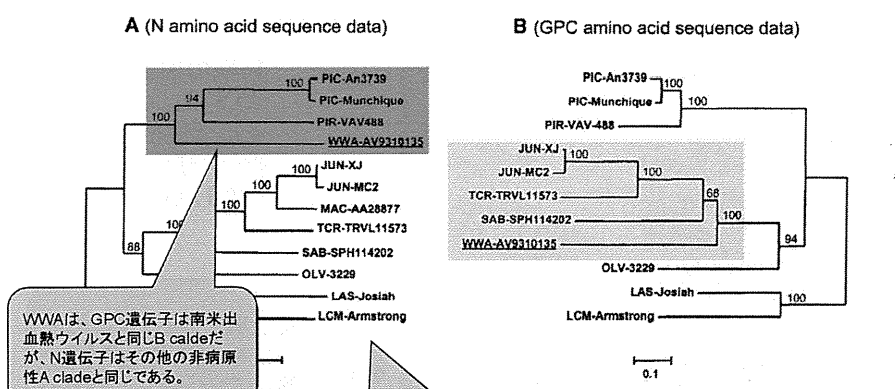
Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia

Simon Delgado[1], Bobbie R. Erickson[2], Roberto Agudo[3], Patrick J. Blair[4], Efrain Vallejo[3], César G. [illegible] Thomas G. Ksiazek[2], James G. Olson[4],





Clade A とB の南米アレナウイルスの組換えによる Whitewater Arroyo virus の出現



WWAは、GPC遺伝子は南米出血熱ウイルスと同じB caldeだが、N遺伝子はその他の非病原性A cladeと同じである。

among arenaviruse[illegible] neighbor-joining analyses of the amino acid sequences of the (A) complete nucleoprotein (N) protein and (B) complete glycoprotein [illegible] gene product. Horizontal branch lengths are proportional to the scale bar; the length of each scale bar represents a sequence dive[illegible] numbers adjacent to the nodes indicate bootstrap support based on an analysis of 500 pseudoreplicate data sets. PIC, Pichindé; [illegible] water Arroyo; JUN, Junin; MAC, Machupo; TCR, Tacaribe; SAB, Sabiá; OLV, Oliveros; LAS, Lassa; and LCM, lym[illegible]

WWAの様な組換えにより新興したウイルスは、日本で整備しているN蛋白を検出する抗原検出系や抗体検出系では対応できない！

新種のエボラウイルスによるアウトブレーク発生（2007-8）

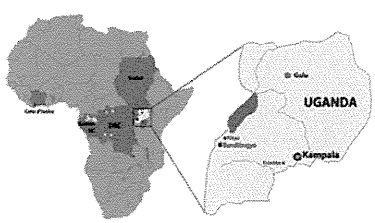
NIID NATIONAL INSTITUTE OF INFECTIOUS DISEASES

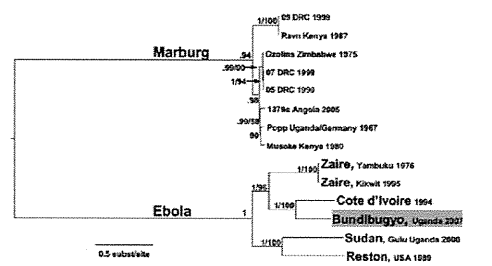
Year	Country	Virus species*	Cases	Deaths	Case fatality(%)
1976	Sudan	Sudan	284	151	53
1976	Zaire (DRC)	Zaire	318	280	88
1977	Zaire (DRC)	Zaire	1	1	100
1979	Sudan	Sudan	34	22	65
1994	Gabon	Zaire	52	31	60
1994	Cote d'Ivoir	Cote d'Ivoir	1	0	0
1995	Liberia	Cote d'Ivoir	1	0	0
1995	DRC	Zaire	315	250	79
1996	Gabon	Zaire	37	21	57
1996 - 1997	Gabon	Zaire	60	45	75
1996	South Africa	Zaire	1 **	1	100
2000 - 2001	Uganda	Sudan	425	224	53
2001 - 2002	Gabon	Zaire	65	53	82
2001 - 2002	DRC	Zaire	59	44	75
2002 - 2003	DRC	Zaire	143	128	90
2003	DRC	Zaire	35	29	83
2004	Sudan	Sudan	17	7	41
2004	Sudan	Sudan	20	5	25
2005	DRC	Zaire	12	9	75
2007	DRC	Zaire	249	183	73
2007 - 2008	Uganda	Bundibugyo	149	37	25
2008-2009	DRC	Zaire	36 ?	12 ?	33
Total			2314	1533	

Ebola-Reston was detected in October 1989 in Reston, Virginia (USA) in a colony of cynomolgus monkeys imported from the Philippines, and in November 1989 in Philadelphia, Pennsylvania, also in monkeys imported from the same supplier. Subsequent outbreaks of Reston-Ebola disease in nonhuman primates occurred in 1990 in the USA (Reston, Virginia and Alice, Texas), in 1992 in Italy (Sienna), and in 1996 in the USA (Alice, Texas). Investigations traced the source of all outbreaks caused by the Reston strain to one export facility in the Philippines (Laguna Province), but the mode of contamination of this facility was not elucidated. **Although highly pathogenic for nonhuman primates, the Reston strain has not to date caused illness in humans.**

A new species of ebolavirus isolated in Ebola HF in Uganda in 2007-8

NIID NATIONAL INSTITUTE OF INFECTIOUS DISEASES



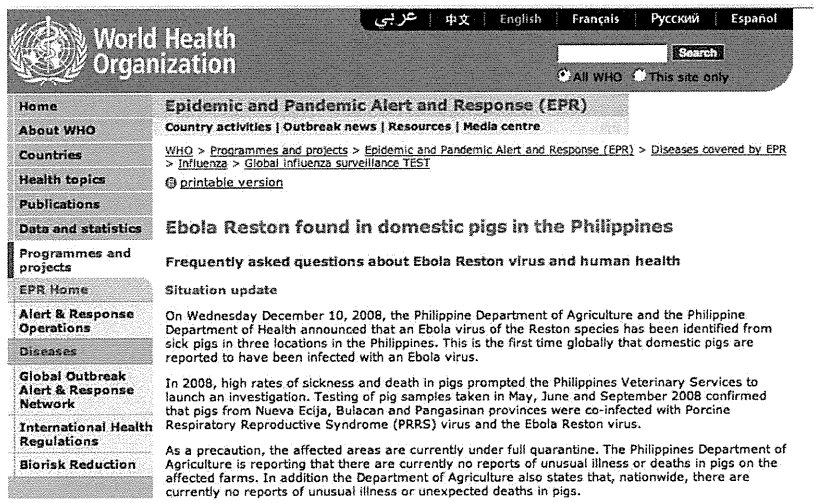


Bundibugyo ebolavirusは、これまでのfilovirus共通RT-PCRで検出できなかった！

ure 1. Geographic locations of Ebola HF outbreaks and phylogenetic relationships of representative filoviruses. (A) Map of Africa owing the sites of all known ebolavirus outbreaks denoted by colored circles for Zaire ebolavirus (yellow), Sudan ebolavirus (green), and Côte d'Ivoire ebolavirus (red). The expanded map of Uganda shows the location of the communities of Bundibugyo and Kibale (black circles) in western Uganda. ... site of the recent outbreak of Bundibugyo ebolavirus. Also shown on the Uganda map are the cities of Kampala (capital), Entebbe (international airport) and Gulu (the site of an outbreak of Sudan ebolavirus in 2000, the largest known Ebola HF outbreak on record). (B) Phylogenetic tree comparing full-length genomes of ebolaviruses and marburgvirus by Bayesian analysis. Posterior probabilities greater than 0.5 and maximum likelihood bootstrap values greater than 50 are indicated at the nodes.
doi:10.1371/journal.ppat.1000212.g001

PLoS Pathogens | www.plospathogens.org 3 November 2008 | Volume 4 | Issue 11 | e1000212

Ebola virus (Reston) infection among pigs in the Philippines in 2008



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Ebola Reston found in domestic pigs in the Philippines

Frequently asked questions about Ebola Reston virus and human health

Situation update

On Wednesday December 10, 2008, the Philippine Department of Agriculture and the Philippine Department of Health announced that an Ebola virus of the Reston species has been identified from sick pigs in three locations in the Philippines. This is the first time globally that domestic pigs are reported to have been infected with an Ebola virus.

In 2008, high rates of sickness and death in pigs prompted the Philippines Veterinary Services to launch an investigation. Testing of pig samples taken in May, June and September 2008 confirmed that pigs from Nueva Ecija, Bulacan and Pangasinan provinces were co-infected with Porcine Respiratory Reproductive Syndrome (PRRS) virus and the Ebola Reston virus.

As a precaution, the affected areas are currently under full quarantine. The Philippines Department of Agriculture is reporting that there are currently no reports of unusual illness or deaths in pigs on the affected farms. In addition the Department of Agriculture also states that, nationwide, there are currently no reports of unusual illness or unexpected deaths in pigs.

新型ウイルス・新興ウイルスへの迅速対応法の構築

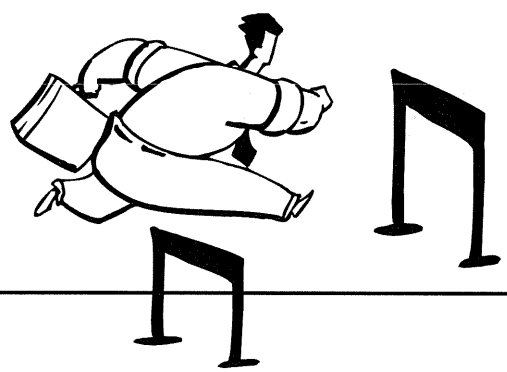
診断法のまとめ

感染症		発生	ウイルス	検査法 ELISA	IF	NT	Ag detection	PCR	LAMP
南米出血熱	アルゼンチン出血熱	Argentine	Junin	NP	NP / GP	VSV-pseudo	NP	(RT-PCR)	
	ボリビア出血熱	Bolivia	Machupo	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
	ベネズエラ出血熱	Venezuela	Guanarito	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
	ブラジル出血熱	Brazil	Sabia	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
	未定	Bolivia	Chapare	(NP)	NP / GP	(VSV-pseudo)	NP	(RT-PCR)	
ラッサ熱		西アフリカ	Lassa	NP	NP / GP	VSV-pseudo	NP	RT-PCR	
未定		Zambia	Lujo	遺伝子組換えの大臣確認実験申請中				(RT-PCR)	
エボラ出血熱		DRC周辺	Zaire ebola	NP / GP	NP / GP	VSV-pseudo	NP	RT-PCR	
		Sudan	Sudan ebola	NP / GP	NP		NP	RT-PCR	
		Uganda	Bundibugyo ebola	(NP)	(NP)		(NP)	(RT-PCR)	
		Côte d'Ivoire	Cote d'Ivoire ebola	NP / GP	NP		NP	RT-PCR	
		Philippines	Reston ebola	NP / GP	NP / GP	VSV-pseudo	NP	RT-PCR	
マールブルグ熱		Angola, Uganda, Kenya, DRC	Lake Victoria Marburg	NP	NP	VSV-pseudo	NP	RT-PCR	LAMP
ニパウイルス感染症		Malasia, Pakistan	Nipah	NP/M/G		(VSV-pseudo)		RT-PCR	
ハンタウイルス肺症候群	北米		Sin Nombre	NP		dNP-ELISA		RT-PCR	
	南米		Andes	NP		dNP-ELISA		RT-PCR	
リフトバレー熱		アフリカ、中東	Rift valley fever	NP, virus	virus	VSV-pseudo, virus	NP	(RT-PCR)	
チクングニア熱		アフリカ、アジア	Chikungunya	virus (IgM)		virus		RT-PCR	
新興ウイルス感染症		**?**	**?**	**遺伝子組換えの大臣確認実験申請では対応が間に合わない**				**CoCoMo primer RDV**	

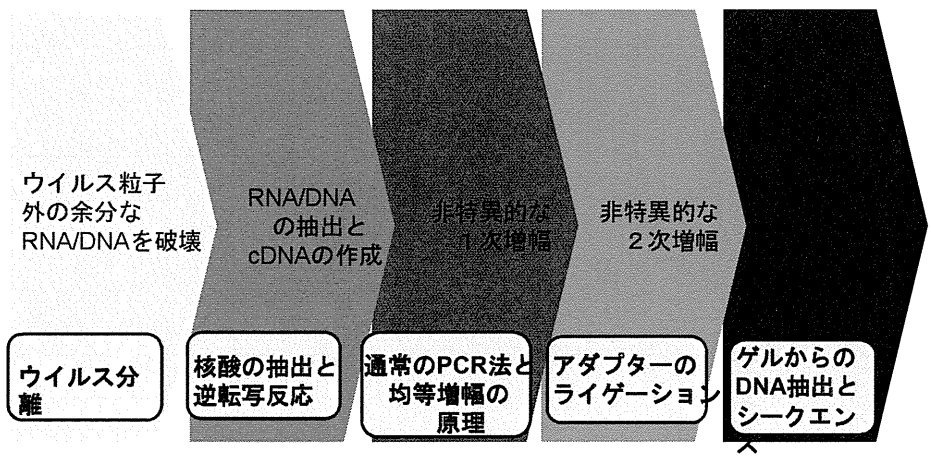
未知のウイルス遺伝子の配列：specific primerを使えない

非特異的な増幅産物を ダイレクトシークエンスする必要がある

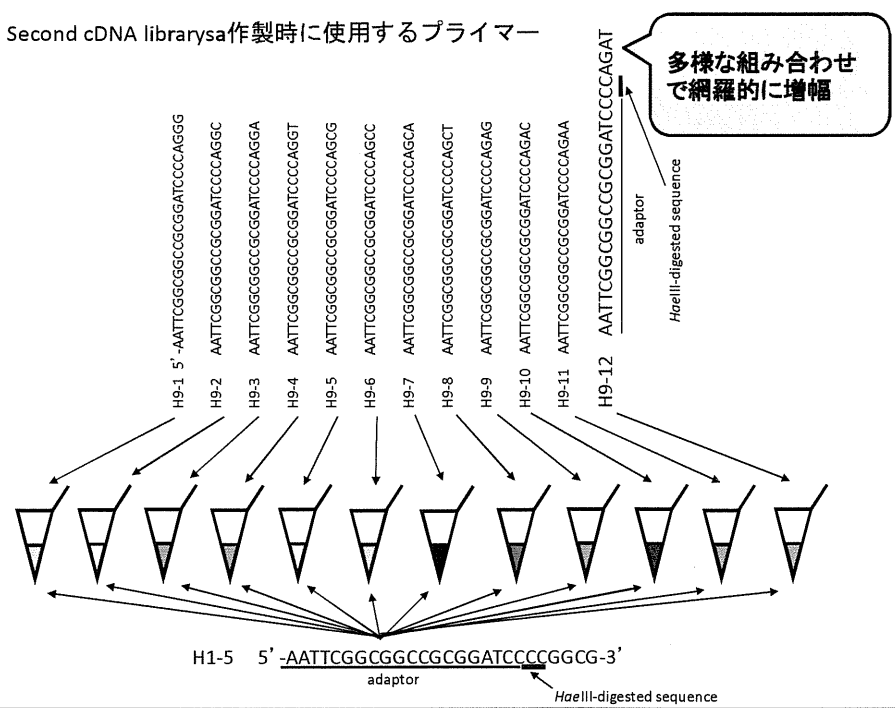
クローニングは大臣確認必要：承認まで時間を要する



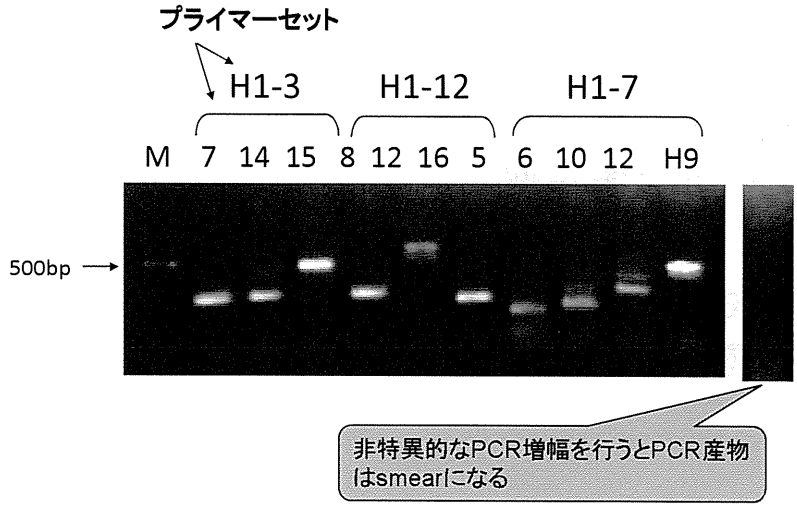
RDV法のステップ



Second cDNA librarysa作製時に使用するプライマー

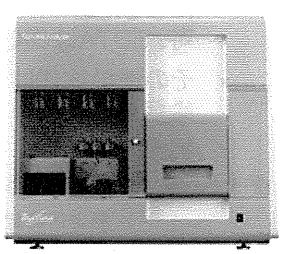


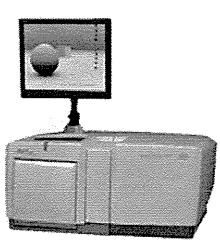
RDV法によるウイルス核酸の最終増幅産物



次世代シークエンサー

Illumina (Solexa): Genome Analyser II	Roche (454 Lifesciences): GS FLX
SBS (Sequencing by synthesis) 600Mbp (6x10^7read)/day	Pyrosequencing 100Mbp(4x10^5read)/day





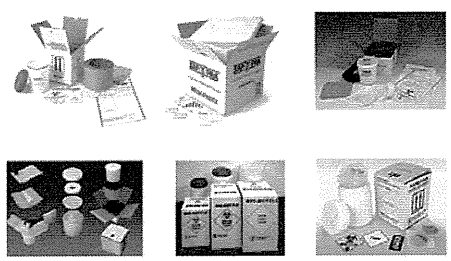
ウイルス性出血熱が疑われる場合の感染研の窓口

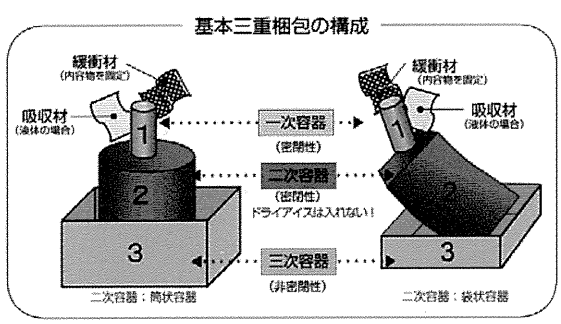
- 感染症情報センター
- ウイルス第一部

（原因不明の感染症が疑われる場合も感染研へ御連絡ください）

検体と輸送法

国連(UN)規格のカテゴリーA容器　サンプル





- ✓ 全血の場合（PCRが陰性になるためヘパリンは不可、EDTAはOK）
- ✓ その他の検体は、疑う感染症による
- ✓ 検査の手続き
  - ➢ 行政検査手続き
    - ➢ 感染研検定係
  - ➢ 検体の輸送法
    - ➢ UN規格カテゴリーA

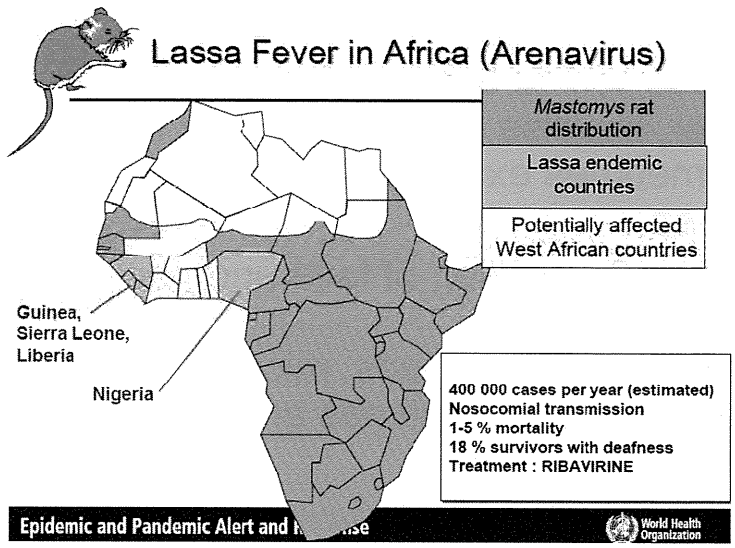

NIID
NATIONAL INSTITUTE OF INFECTIOUS DISEASES

Viral haemorrhagic fevers: risk assessment and clinical care

Dr Barbara Bannister
Royal Free Hospital

Arenavirus family

- Old-world: Lassa, which causes sporadic and epidemic cases in West Africa; Lujo, a virus originating in Zambia, which caused an outbreak of five cases and four deaths in 2008
- New world: Junin (Argentina), Machupo (Bolivia), Guanarito (Venezuela) and Sabia (Brazil)
- Many arenaviruses non-pathogenic to humans (eg Moipea) are recognised in rodents, especially in Africa



Lassa viruses

- Four lineages: I-III Nigerian and VI, the Josiah strain from Sierra Leone, which is very similar to isolates from Liberia and Guinea
- The three Nigerian lineages show large sequence-differences in NP and GP-1genes; up to 27% nucleic acid and 15% amino-acid divergence (*Bowen MD et al. J virol 2000: 74: 6992*)

Clinical features of Lassa fever

- Phase 1: 7 to 8 days of fever, malaise, headache, sore throat (sometimes exudative tonsillitis), muscle aches
- Phase 2: swelling of face and neck, slight fall in blood pressure, nosebleeds, microscopic haematuria, sometimes encephalopathy or nerve deafness
- Phase 3 after 10-12 days: shock, sepsis, multi-organ failure (severe haemorrhage may not occur, but indicates

Differential diagnosis of Lassa fever

- Malaria (do not neglect this treatable condition)
- Common infections, eg urinary infections, acute pharyngitis or tonsillitis, HIV seroconversion
- many viral infections, including hepatitis A, hepatitis E, yellow fever,
- Typhoid fever
- Rickettsial infections

Laboratory investigations 1

- Phase 1: moderate rise in C-reactive protein, low or normal white cell count, usually normal haemoglobin, liver function tests and coagulation tests
- Phase 2: Aspartate transaminase rises (may reach 400-2000 u/l and peaks for about three days); alanine transaminase shows much less elevation; SpO2 ;may fall below 95%; platelet count falls moderately; pancreatic lipase and muscle enzymes (eg creatine kinase) may rise

Laboratory investigations 2

- Phase 3: oxygen saturation falls quickly, platelet count falls rapidly, creatinine rises with onset of renal failure, electrolyte levels may alter as major capillary leak occurs, blood albumin falls rapidly
- Patient management lab tests now focus on managing the sepsis syndrome and detecting secondary conditions such as urinary infection and ventilator-associated pneumonia