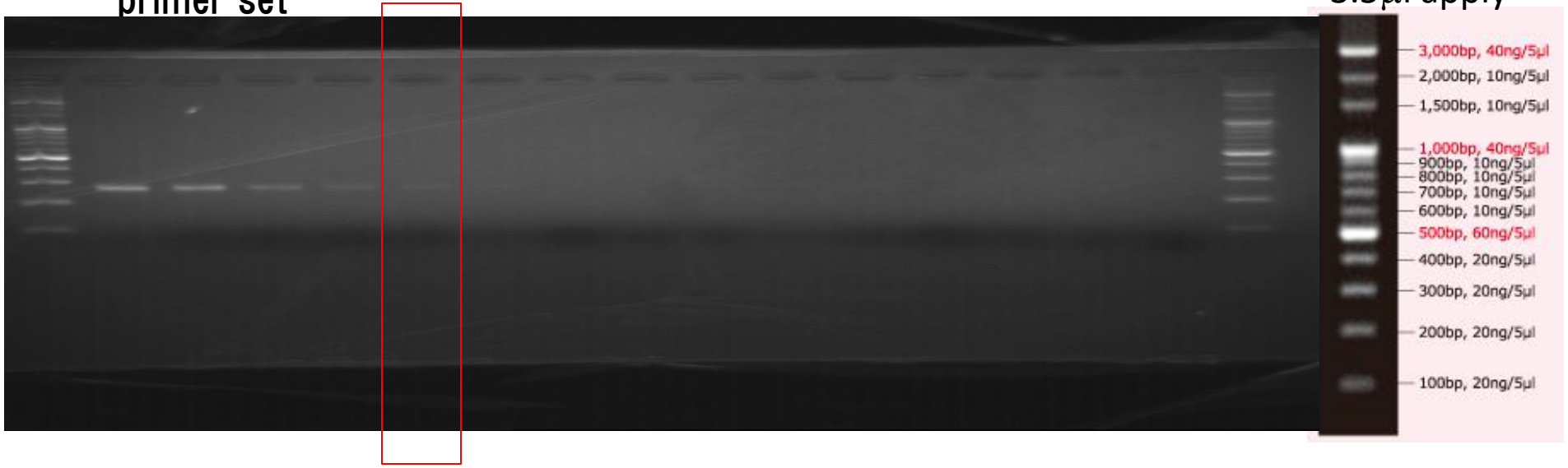
STH-PAS による特定遺伝子の検出

(Single strand Tag Hybridization –Printed Strip Array)

アガロースゲル電気泳動/EtBr 検出感度

Results of electrophoresis

Sample: Bovine DNA(50ng) was used for amplification using PAS primer set



Signal intensity of ③was judged to be equivalent to the signal intensity of 300bp band of 100bp ladder markers (14ng).

DNA amounts in lanes

①:56ng	⑥:1.75ng	⑪:0.0515625ng
②:28ng	⑦:0.825ng	⑫:0.02578125ng
③:14ng	⑧:0.4125ng	⑬:0.012890625ng
④:7ng	⑨:0.20625ng	⑭:0.0064453125ng
⑤:3.5ng	⑩:0.103125ng	⑮:0.00322265625ng

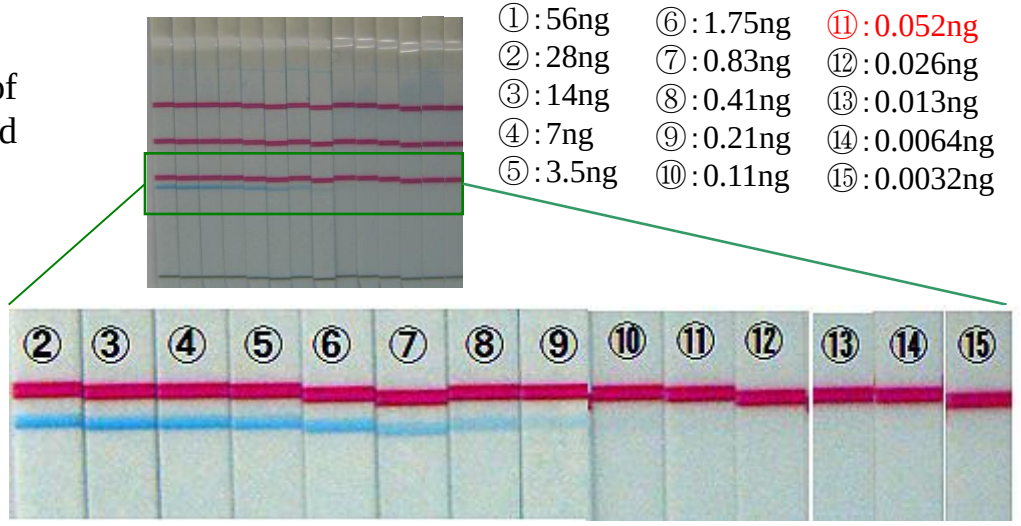
Detection limit is 3.5ng DNA (lane ⑤)

STH-PAS 検出感度

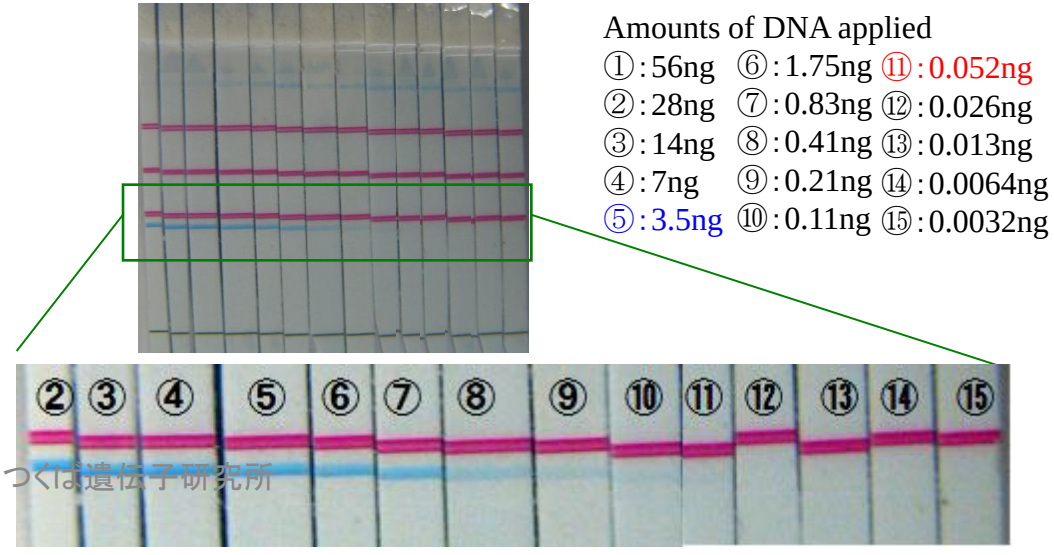
Experiments

・PCR products, which were used in the test of electrophresis/EtBr detection sensitivity, were used for the test of PAS detection sensitivity.

Results：①ver. 1 PAS



②ver.2 Primer trap-PAS

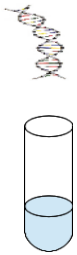


Detection limit of PAS： ver1 and ver2 showed that the limit is **0.052ng** ⑪

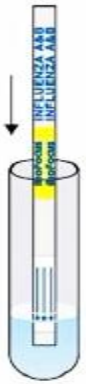
STH-PAS 検出の流れ

Biological sample

Extract DNA/RNA



PCR products



Place the test strip into the test tube

Wait for 5 min

Genuine detection

target 2

Target 1

Positive control

PCR using specific primers of different target to detect wide range of strains in a single PCR

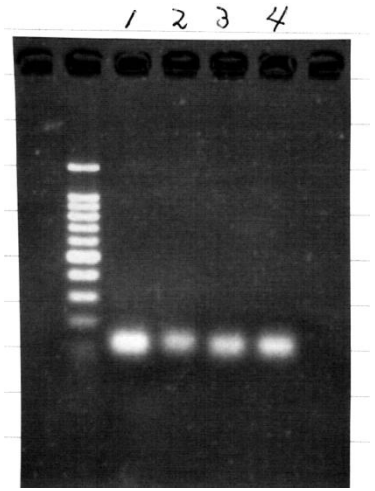
Norovirus, sometimes known as the **winter vomiting bug**[1] in the UK, is the most common cause of viral gastroenteritis in humans. It affects people of all ages.[2] The virus is transmitted by fecally contaminated food or water, by person-to-person contact,[3] and via aerosolization of the virus and subsequent contamination of surfaces.[4] The virus affects around 267 million people and causes over 200,000 deaths each year; these deaths are usually in less developed countries and in the very young, elderly and immunosuppressed.[5]

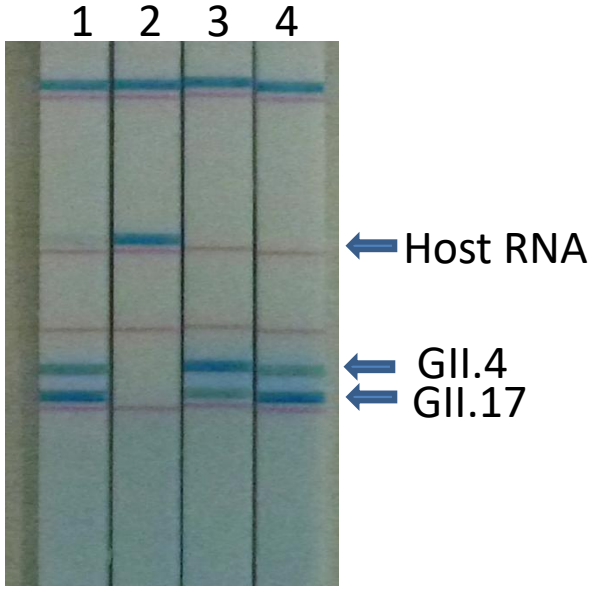
Noroviruses (NoV) are a genetically diverse group of single-stranded RNA, non-enveloped viruses belonging to the *Caliciviridae* family.[25] According to the International Committee on Taxonomy of Viruses, the genus *Norovirus* has one species, which is called *Norwalk virus*.[26] Noroviruses can genetically be classified into five different genogroups (GI, GII, GIII, GIV, and GV), which can be further divided into different genetic clusters or genotypes. For example, genogroup II, the most prevalent human genogroup, presently contains 19 genotypes. Genogroups I, II and IV infect humans, whereas genogroup III infects bovine species, and genogroup V has recently been isolated in mice.[28] g

In the present project, the **36** complete sequences of human norovirus (all of the sequences registered at NCBI at 2015/02/22 14:01) were collected and used for determination of detection primer.

Examination with host materials

1. Human RNA +GII.4 +GII.17
2. Human RNA
3.Human RNA+ GII.4
4. Human RNA+ GII.17





Detection with lower numbers of norovirus RNA moleclues

PCR: 33 cycles

1: **100** molecules/RT-reaction
2: **10** molecules/RT-reaction
3: water in RT/ reaction
4: water in PCR (noRT)

is supposed to be a threshold for regular detection

Zika virus outbreak (2015–present)

As of early 2016, a widespread outbreak of Zika fever, caused by the Zika virus, is ongoing, primarily in the Americas. The outbreak began in April 2015 in Brazil, and has spread to other countries in South America, Central America, Mexico, and theCaribbean. In January 2016, the World Health Organization (WHO) said the virus was likely to spread throughout most of the Americas by the end of the year;[2] and in February 2016, the WHO declared the cluster of microcephaly and Guillain–Barré syndrome (GBS) cases reported in Brazil – strongly suspected to be associated with the Zika virus outbreak – a Public Health Emergency of International Concern.[3][4][5][6]

The Zika virus was first linked with newborn microcephaly during the Brazil Zika virus outbreak. In 2015, there were 2,782 cases of microcephaly compared with 147 in 2014 and 167 in 2013.[68]

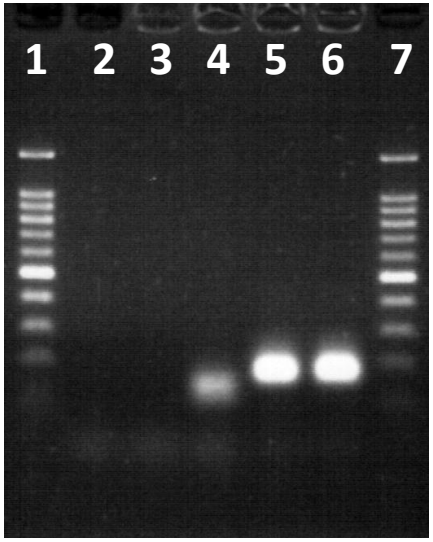
Zika is a mosquito-borne disease and possibly a sexually transmitted infection.[8] The resurgence of Aedes aegypti's worldwide distribution over the past 2–3 decades makes it one of the most widely distributed mosquito species.[63] In 2015, Aedes albopictus was present in tropical, subtropical, and temperate regions of the Americas, reaching as far north as the Great Lakes of North America and, internationally, living alongside Aedes aegypti in some tropical and subtropical regions.[7]

Twelve Zika virus complete genome sequences were found in NCBI nucleotide database at point of 10th February, 2016. Therefore, all the virus sequences were collected and primer set was designed to detect all virus sequences registered at this time point.

Procedures

1. RT using Zika specific RT-primer and mammalian rRNA specific RT-primer
2. PCR using respective species specific primer pairs
3. Electrophoresis using a 2.5% agarose gel.

1,7:100bp marker
2: H2O at RT
3: H2o at PCR
4: Human RNA
5:Zika RNA(portion)
6:Zika RNA(portion) plus HumanRNA



PAS解析

Conclusion : Zika viruses are able to be detected

Dengue virus

Dengue fever (UK /ˈdɛŋgeɪ/ or US /ˈdɛŋgiː/), also known as breakbone fever, is a mosquito-borne tropical disease caused by the dengue virus consisting of single positive-strand RNA.

The dengue virus genome (genetic material) contains about 11,000 nucleotide bases, which code for the three different types of protein molecules (C, prM and E) that form the virus particle and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are only found in infected host cells and are required for replication of the virus.[15][17] There are five[1] strains of the virus, called serotypes, of which the first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4.[4] The fifth type was announced in 2013.[1] http://en.wikipedia.org/wiki/Dengue_fever

Currently, we are focusing on the detection of DENV-1, DENV-2, DENV-3 and DENV-4

Collection of dengue virus complete genome sequence

Type 1: 49 genome sequences

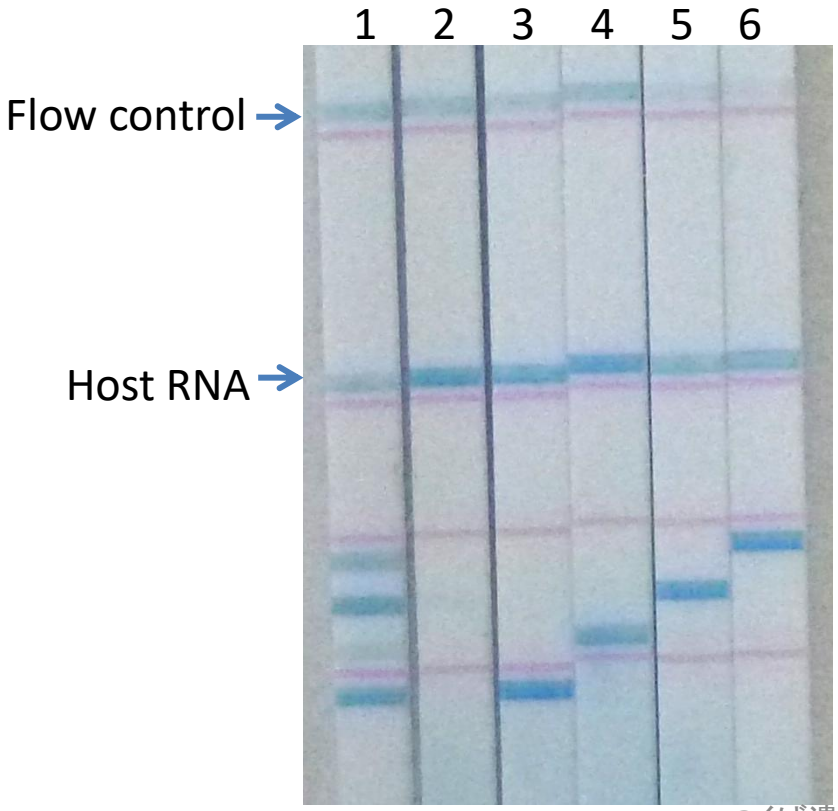
Type2: 50 genome sequences

Type3: 50 genome sequences

Type4: 50 genome sequences

PAS system test with host materials 2

	1	2	3	4	5	6
RT-Primer	Mix-Hu	Mix-Hu	Mix-Hu	Mix-Hu	Mix-Hu	Mix-Hu
RNA	Mix50	Hu50	D1-50, Hu50	D2-50, Hu50	D3-50, Hu50	D4-50, Hu50
	↓	↓	↓	↓	↓	↓
cDNA	AM-AM	AM-H	AM-1H	AM-2H	AM-3H	AM-4H
M.P. Primer	[F-X], [F-8]	[F-X], [F-8]	[F-X], [F-8]	[F-X], [F-8]	[F-X], [F-8]	[F-X], [F-8]
Size(bp)	Mix	105	130, 105	142, 105	153, 105	126, 105



3: Type 1: 49 genome sequences

4: Type2: 50 genome sequences

5: Type3: 50 genome sequences

5: Type4: 50 genome sequences

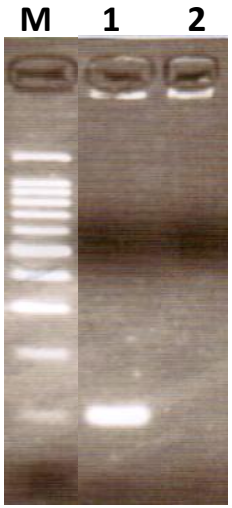
Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a group of single-celled microorganism) belonging to the genus *Plasmodium*.Malaria causes symptoms that typically include fever, fatigue, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma or death.

Five species of *Plasmodium* can infect and be spread by humans.[2] Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria.[1][2] The species *P. knowlesi* rarely causes disease in humans.[1]

From wikipedia

Detection of malaria 5 species rRNA with Mix primer (multiplex RT and PCR)

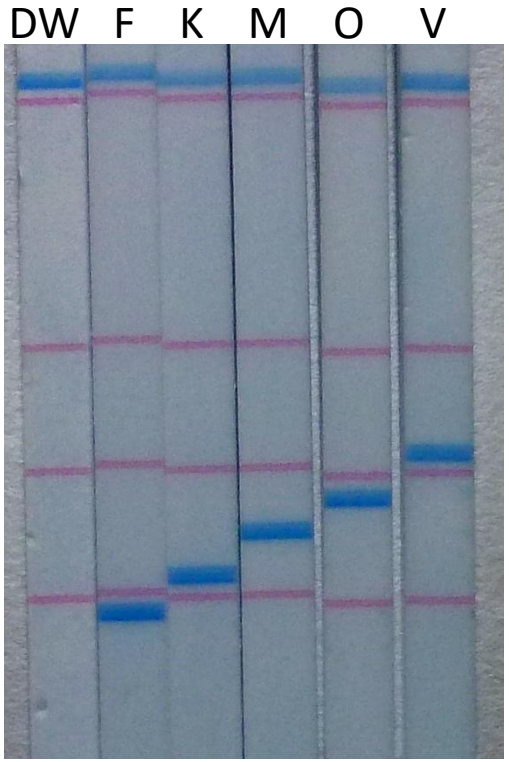
STH-PAS detection using 1μl of PCR product



M, molecular weight marker
1, Human RNA human rRNA RT and PCR primer
2, Human RNA Maralia RT and PCR primers

Malaria signal was not detected in RNA from non-infected human cells (see lane2)

For detailed examination, see next page



熱帯熱マラリア原虫（*P. falciparum*）、
三日熱マラリア原虫（*P. vivax*）、
四日熱マラリア原虫（*P. malariae*）、
卵形マラリア原虫（*P. ovale*）
サルマラリア原虫（*P. knowlesi*）

Ebola virus disease

Ebola virus disease (**EVD**; also **Ebola hemorrhagic fever**, or **EHF**), or simply **Ebola**, is a disease of humans and other primates caused by ebolaviruses. Signs and symptoms typically start between two days and three weeks after contracting the virus with a fever, sore throat, muscular pain, and headaches. Then, vomiting, diarrhea and rash usually follow, along with decreased function of the liver and kidneys. At this time some people begin to bleed both internally and externally.[1] The disease has a high risk of death, killing between 25 and 90 percent of those infected, with an average of about 50 percent.[1]

Ebolaviruses contain single-stranded, non-infectious RNA genomes.[29] *Ebolavirus* genomes contain seven genes including 3‘-UTR-*NP-VP35-VP40-GP-VP30-VP24-L*-5’-UTR.[21][30] The genomes of the five different ebolaviruses (BDBV, EBOV, RESTV, SUDV and TAFV) differ in sequence

The natural reservoir for Ebola has yet to be confirmed; however, bats are considered to be the most likely candidate species.[43]

http://en.wikipedia.org/wiki/Ebola_virus_disease

Collected viral genome sequences

Sudan ebolavirus
http://www.ncbi.nlm.nih.gov/nuccore/55770807
LOCUS NC_006432 18875 bp cRNA linear VRL 12-NOV-2014
All (10 seqs) the complete genome sequences registered on 2014.12.31

Reston ebolavirus
http://www.ncbi.nlm.nih.gov/nuccore/22789222
LOCUS NC_004161 18891 bp cRNA linear VRL 12-NOV-2014
All (8 seqs) the complete genome sequences registered on 2014.12.31

Zaire ebolavirus
http://www.ncbi.nlm.nih.gov/nuccore/10313991
LOCUS NC_002549 18959 bp cRNA linear VRL 12-NOV-2014
11 complete genome sequences randomly-selected from the sequences registered on 2014.12.31

Bundibugyo virus
http://www.ncbi.nlm.nih.gov/nuccore/NC_014373
LOCUS NC_014373 18940 bp cRNA linear VRL 12-NOV-2014
All (6 seqs) the complete genome sequences registered on 2014.12.31

Tai Forest ebolavirus
http://www.ncbi.nlm.nih.gov/nuccore/302315369
LOCUS NC_014372 18935 bp cRNA linear VRL 12-NOV-2014
All (2 seqs) the complete genome sequences registered on 2014.12.31

Lloviu cuevavirus
http://www.ncbi.nlm.nih.gov/nuccore/355469071
LOCUS NC_016144 18927 bp cRNA linear VRL 12-NOV-2014
All (2 seqs) the complete genome sequences registered on 2014.12.31

Marburg marburgvirus
http://www.ncbi.nlm.nih.gov/nuccore/158539108
LOCUS NC_001608 19111 bp cRNA linear VRL 12-NOV-2014
10 complete genome sequences randomly-selected from the sequences registered on 2014.12.31

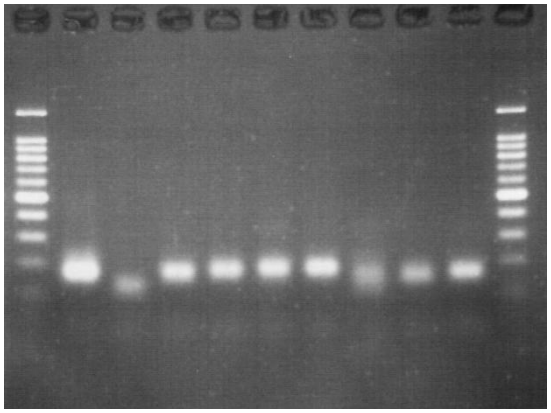
Examination of PAS system for ebola virus RNA (Ebola5+2) under the pre-existing host RNA

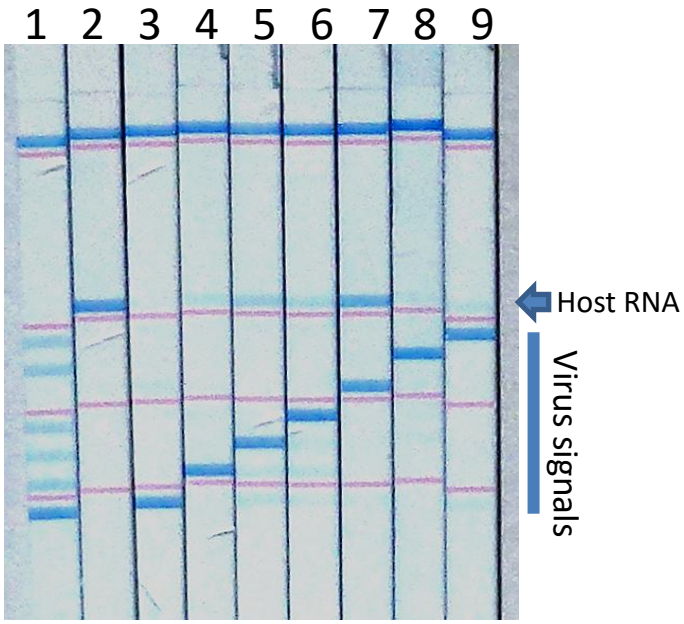
Trial 3 (final condition)

Condition: RT65C 60min, 70C 15min

PCR 95C 9min(1cycle), 95C 30sec, 68C 30sec, 72C 30sec (28cycles), 72C 5min(1cycle)

	1	2	3	4	5	6	7	8	9
RT-Primer	Mix-RT, Hu-RT	Mix-RT, Hu-RT	Mix-RT, Hu-RT	Mix-RT, Hu-RT	Mix-RT, Hu-RT	Mix-RT, Hu-RT	Mix-RT, Hu-RT	Mix-RT, Hu-RT	Mix-RT, Hu-RT
RNA	Mix150,50	Hu150	B50, Hu150	L50, Hu150	M50, Hul50	R50, Hu150	S50, Hu150	T50, Hu150	Z50, Hu150
	↓	↓	↓	↓	↓	↓	↓	↓	↓
cDNA	AM-AM	AM-H	AM-BH	AM-LH	AM-MH	AM-RH	AM-SH	AM-TH	AM-ZH
M.P. Primer	[F-X] [F-8]	[F-X] [F-8]	[F-X] [F-8]	[F-X] [F-8]	[F-X] [F-8]	[F-X] [F-8]	[F-X] [F-8]	[F-X] [F-8]	[F-X] [F-8]
Size(bp)	Posi	105	141, 105	154, 105	149, 105	156, 105	143, 105	141, 105	139, 105





1. **Virus RNA mix**
2. **Host RNA only (Human)**
3. **Bundibugyo virus + hostRNA**
4. **Lloviu cuevavirus + hostRNA**
5. **Marburg marburgvirus + hostRNA**
6. **Reston ebolavirus + hostRNA**
7. **Sudan ebolavirus + hostRNA**
8. **Tai Forest ebolavirus + hostRNA**
9. **Zaire ebolavirus + hostRNA**

Middle East respiratory syndrome coronavirus

From Wikipedia, the free encyclopedia

(Redirected from MERS-CoV)

This article is about the virus. For the disease, see Middle East respiratory syndrome. For the outbreak, see 2012 Middle East respiratory syndrome coronavirus outbreak.

MERS-CoV

MERS-CoV particles as seen by negative stain electron microscopy. Virions contain characteristic club-like projections emanating from the viral membrane.

Virus classification

Group: Group IV ((+)ssRNA)
Order: Nidovirales
Family: Coronaviridae
Subfamily: Coronavirinae
Genus: Betacoronavirus
Species: MERS-CoV

As of 16 April 2014, MERS-CoV cases have been reported in several countries, including Saudi Arabia, Malaysia, Jordan, Qatar, the United Arab Emirates, Tunisia, and the Philippines. The MERS count is 238, with 92 deaths[2] (see 2012 Middle East respiratory syndrome coronavirus outbreak for a full list of countries and casualities).

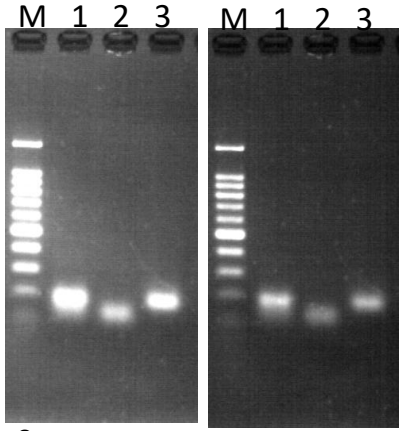
PAS system test with host materials

Condition (PCR machine #2) RT:65C
95C 9min (1cycle), 95C 30sec **68C** 30sec 72C 30sec (28cycles), 72C 5min (1cycle)

	1	2	3
RT-Primer	Sars2, HumanN2	Sars2, HumanN2	Sars2, HumanN2
RNA	Sars2N, Human	Human	Sars2N
	↓	↓	↓
cDNA	AM-AM	AM-H	AM-S
M.P. Primer	[F-3] [F-8]	[F-3] [F-8]	[F-3] [F-8]
Size(bp)	Posi	80+25 (105)	133+25 (158)

Sars2N: Mers RNA* 50ng
Human: humal RNA 50ng

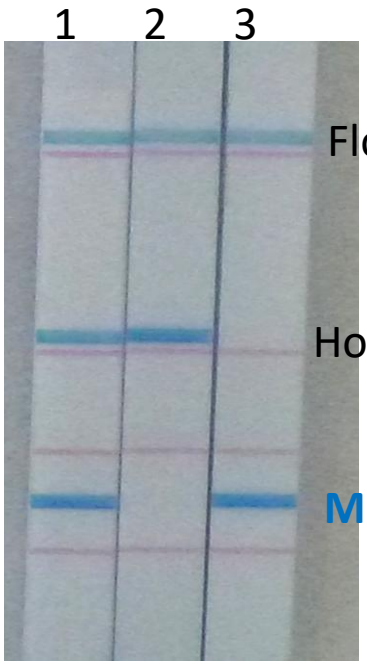
* Partial sequence of **MERS –CoV** RNA



Over exposure

Electrophoresis

Based on the electrophoresis, the system works fine.



Detection method finalized

Detection of HEV RNA with Printed Strip Array(PAS)

Nested PCR for Genotype 1, G2, G3, G4

Reverse transcription

↓

PCR

↓ 1hr

PAS detection

5-6min

1:control (no virus RNA)

2: HEV RNA +

Signal of HEV RNA

つくば遺伝子研究所