Novel coronavirus associated with severe respiratory disease - Laboratory aspects

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Subject: PRO/EDR> Novel coronavirus - Saudi Arabia: human isolate
Archive Number: 20120920.1302733

NOVEL CORONAVIRUS - SAUDI ARABIA: HUMAN ISOLATE
****

A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
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http://www.isid.org

Date: Sat 15 Sep 2012

From: Ali Mohamed Zaki <azaki53@hotmail.com> [edited]

Background

- 20 September 2012: Isolation of novel coronavirus from patient in Saudi Arabia
- M/60 years: Pneumonia associated with acute renal failure
- Virus grows readily on Vero and LLC-MK2 cells: Rounding and syncytia formation
- Pan-coronavirus RT-PCR: Positive
- Netherlands laboratory: Confirmed new member of beta group of corononavirus closely related to bat coronaviruses

Further information

- Eurosurveillance 27 September 2012
  - Saudi Arabia case reported in Promed:
    - 13 June 2012: Presented with pneumonia
    - 24 June 2012: Passed away
  - Qatar case:
    - M/40+
    - 11 September 2012: Evacuated to United Kingdom for management of pulmonary and renal failure
    - Pan-coronavirus PCR positive for coronavirus; nucleotide sequence closely matched with virus from above case

Published Date: 2012-11-04 13:11:42
Subject: PRO/AH/EDR> Novel coronavirus - Saudi Arabia (15): new case
Archive Number: 20121104.1391285

NOVEL CORONAVIRUS - SAUDI ARABIA (15): NEW CASE
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A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Nov 4, 2012 12:11 PM

From: Ziad Memish <zmemish@yahoo.com>

Subject: Re: A new Saudi novel coronavirus case diagnosed in KSA (Kingdom of Saudi Arabia)

Novel coronavirus infection - update

23 NOVEMBER 2012 - WHO has been notified of four additional cases, including one death, due to infection with the novel coronavirus. The additional cases have been identified as part of the enhanced surveillance in Saudi Arabia (3 cases, including 1 death) and Qatar (1 case). This brings the total of laboratory confirmed cases to 6.

Investigations are ongoing in areas of epidemiology, clinical management, and virology, to look into the likely source of infection, the route of exposure, and the possibility of human-to-human transmission of the virus. Close contacts of the recently confirmed cases are being identified and followed-up.

So far, only the two most recently confirmed cases in Saudi Arabia are epidemiologically linked - they are from the same family, living in the same household. Preliminary investigations indicate that these 2 cases presented with similar symptoms of illness. One died and the other recovered.

Additionally, 2 other members of this family presented with similar symptoms of illness, where one died and the other is recovering. Laboratory results of the fatal case is pending, while the case that is recovering tested negative for the novel coronavirus.

Related links

More information on coronavirus infections

Outbreak news on coronavirus infections

Case definition for case finding severe respiratory disease associated with novel coronavirus

Information regarding requirements and recommendations for the Hajj season in 2012

Cases 3-6

- Case 3:
  - Saudi Arabia
  - Recovered
- Case 4:
  - Qatar
  - Not fatal
- Case 5 and 6:
  - Saudi Arabia
  - Epidemiologically linked (family members)
  - One fatal, one recovered

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Novel coronavirus infection - update

30 NOVEMBER 2012 - In addition to the fatal case of novel coronavirus in Saudi Arabia reported to WHO on 28 November, two fatal cases in Jordan have been reported to WHO today, bringing the total of laboratory-confirmed cases to nine.

The latest confirmed case from Saudi Arabia occurred in October 2012 and is from the family cluster of the two cases confirmed earlier.

The two cases from Jordan occurred in April 2012. At that time, a number of severe pneumonia cases occurred in the country and the Ministry of Health (MOH) Jordan promptly requested a WHO Collaborating Centre for Emerging and Re-emerging Infectious Diseases (NAMRU – 3) team to immediately assist in the laboratory investigation. The NAMRU-3 team went to Jordan and tested samples from this cluster of cases.

On 24 April 2012 the NAMRU-3 team informed the MOH that all samples had tested negative for known coronaviruses and other respiratory viruses. As the novel coronavirus had not yet been discovered, no specific tests for it were available.

In October 2012, after the discovery of the novel coronavirus, stored samples were sent by MOH Jordan to NAMRU-3. In November 2012 NAMRU-3 provided

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Related links

More information on coronavirus infections
Outbreak news on coronavirus infections
Case definition for case finding severe respiratory disease associated with novel coronavirus
Information regarding requirements and recommendations for the Hajj season in 2012
Background and summary of novel coronavirus infection – as of 30 November 2012

Cases 7-9

- Case 7:
  - Saudi Arabia
  - Occurred in October 2012
  - Epidemiologically linked to cases 5 and 6
  - Fatal
- Case 8-9:
  - Jordan
  - April 2012
  - Epidemiologically linked to each other
  - Both fatal

European CDC Communicable Disease Threats Report

Severe respiratory disease of unknown origin – Jordan - Outbreak in ICU

Opening date: 26 April 2012 Latest update: 3 May 2012

Epidemiological summary

An outbreak of a respiratory illness was reported on 19 April 2012 by the Ministry of Health in an intensive care unit in a hospital in Zarqa, Jordan. Seven nurses and one doctor were among the 11 affected. One of the nurses died. Jordan's Ministry of Health acknowledges the fatal case specifying she had underlying conditions and all cases had high fever and lower respiratory symptoms. According to the Ministry, the origin of the infection is likely to be viral. However, laboratory results are not available to date.

ECDC assessment

ECDC is following this event due to its severity including one fatality and the unusualness of the disease affecting health care staff. These cases drew high media attention this week.

Actions

ECDC contacted both Episouth, WHO and US CDC for further information. Both WHO and US CDC are following this event

Cases 8 and 9

- April 2012: A number of severe pnemonia cases in Jordan
- WHO Collaborating Centre for Emerging and Re-emerging Infectious Diseases (NAMRU – 3) provided testing: All samples negative for known coronaviruses/other respiratory viruses
- October 2012: Stored samples sent by Jordan to NAMRU-3, confirmed two novel coronavirus infection cases in November

Summary

- First known case infected in April 2012 (Jordan)
- So far confined to Middle East
  - Jordan: 2/2 fatal
  - Qatar: 0/2 fatal
  - Saudi Arabia: 3/5 fatal
- Evidence of limited person-to-person transmission

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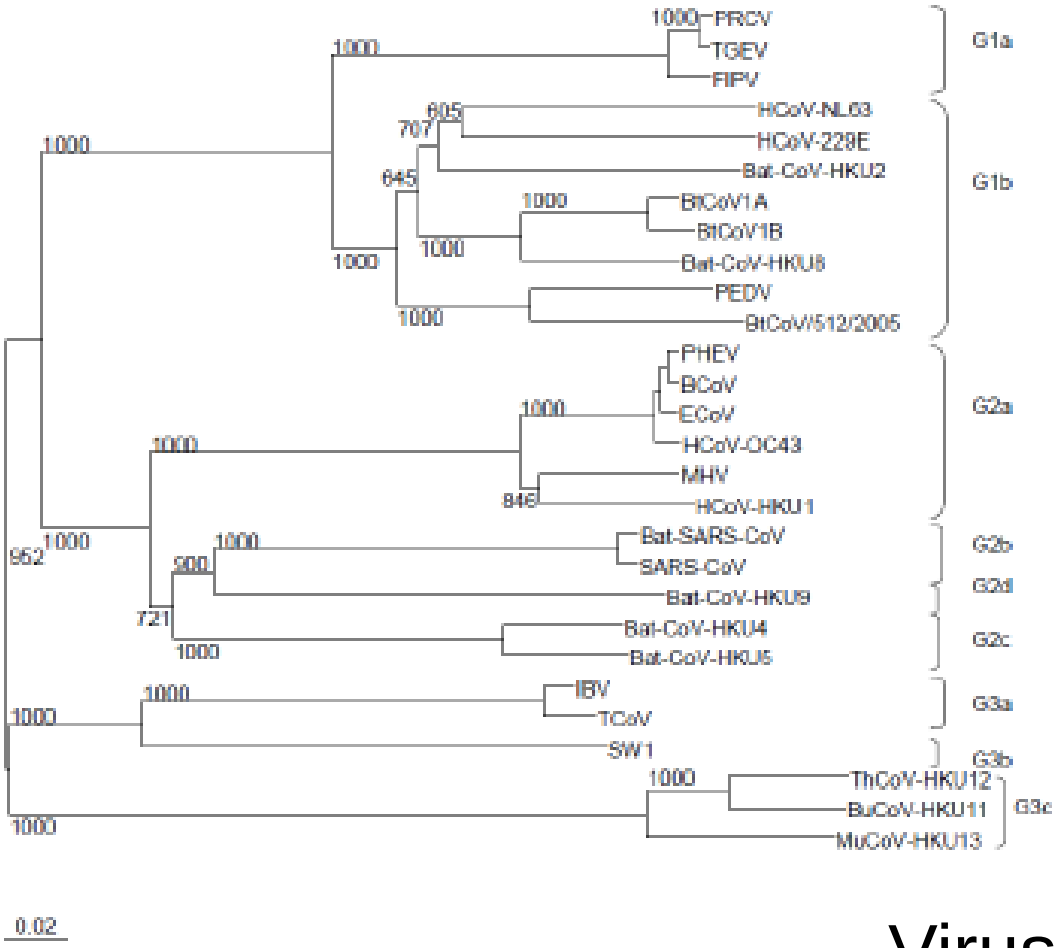
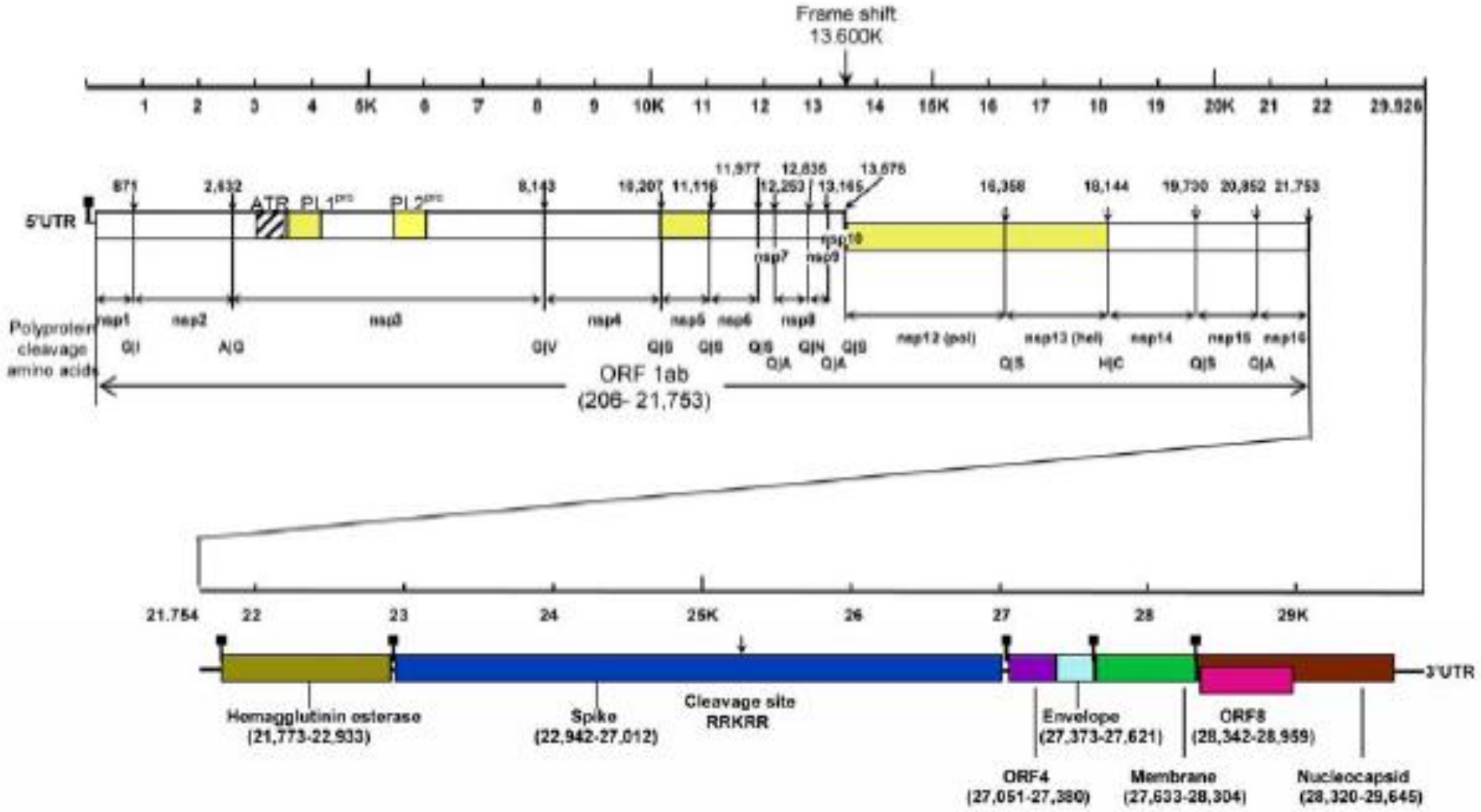
Figure 1. Phylogenetic analysis of RNA-dependent RNA polymerases of coronaviruses with complete genome sequences available by the end of 2008. The tree was constructed by neighbor joining method using Kimura's two-parameter correction and bootstrap values calculated from 1000 trees. Nine hundred and fifty eight amino acid positions were included in the analysis. The scale bar indicates the estimated number of substitutions per 50 amino acids.



Virus 2009; 1: 57


Family: Coronaviridae (2 Subfamilies)
Subfamily: Coronavirinae (4 Genera)
Genus: Alphacoronavirus (8 Species)
Species: Alphacoronavirus 1
Species: Human coronavirus 229E
Species: Human coronavirus NL63
Species: Miniopterus bat coronavirus 1
Species: Miniopterus bat coronavirus HKU8
Species: Porcine epidemic diarrhea virus
Species: Rhinolophus bat coronavirus HKU2
Species: Scotophilus bat coronavirus 512
Genus: Betacoronavirus (7 Species)
Species: Betacoronavirus 1
Species: Human coronavirus HKU1
Species: Murine coronavirus
Species: Pipistrellus bat coronavirus HKU5
Species: Rousettus bat coronavirus HKU9
Species: Severe acute respiratory syndrome-related coronavirus
Species: Tylonycteris bat coronavirus HKU4
Genus: Deltacoronavirus (3 Species)
Species: Bulbul coronavirus HKU11
Species: Munia coronavirus HKU13
Species: Thrush coronavirus HKU12
Genus: Gammacoronavirus (2 Species)
Species: Avian coronavirus
Species: Beluga whale coronavirus SW1
Subfamily: Torovirinae (2 Genera)



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Virus 2009; 1: 57

Diagnostic strategy – Initial

- Pan-coronavirus reverse-transcription polymerase chain reaction
- Nucleotide sequencing
- Sequence analysis
- Turnaround time:
  - Negative – 1 day
  - Positive – 2 days

```
LOCUS       JX869059               30118 bp    RNA     linear   VRL 27-SEP-2012
DEFINITION  Human betacoronavirus 2c EMC/2012, complete genome.
ACCESSION   JX869059
VERSION     JX869059.1  GI:407076736
KEYWORDS    .
SOURCE      Human betacoronavirus 2c EMC/2012
  ORGANISM  Human betacoronavirus 2c EMC/2012
            Viruses; ssRNA positive-strand viruses, no DNA stage; Nidovirales;
            Coronaviridae; Coronavirinae; Betacoronavirus; unclassified
            Betacoronavirus.
REFERENCE   1  (bases 1 to 30118)
  AUTHORS   van Boheemen,S., Zaki,A.M., Bestebroer,T.M., de Graaf,M.,
            Victor,S., Osterhaus,A.D., Haagmans,B.L. and Fouchier,R.A.
  TITLE     Genomic Analysis for a Newly Isolated Human Betacoronavirus Lineage
            2C
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 30118)
  AUTHORS   van Boheemen,S., Zaki,A.M., Bestebroer,T.M., de Graaf,M.,
            Victor,S., Osterhaus,A.D., Haagmans,B.L. and Fouchier,R.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-SEP-2012) Viroscience Lab, Erasmus MC, Dr.
            Molewaterplein 60, Rotterdam, Zuid-Holland 3000CA, The Netherlands
COMMENT     [WARNING] On Oct 16, 2012 this sequence was replaced by
            gi:409052551.
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RAPID COMMUNICATIONS

Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction

V M Corman[1,2], I Eckerle[1], T Bleicker[1], A Zaki[3], O Landt[4], M Eschbach-Bludau[1], S van Boheemen[5], R Gopal[6], M Ballhause[4], T M Bestebroer[5], D Muth[1], M A Müller[1], J F Drexler[1], M Zambon[6], A D Osterhaus[5], R M Fouchier[5], C Drosten (drosten@virology-bonn.de)[1]

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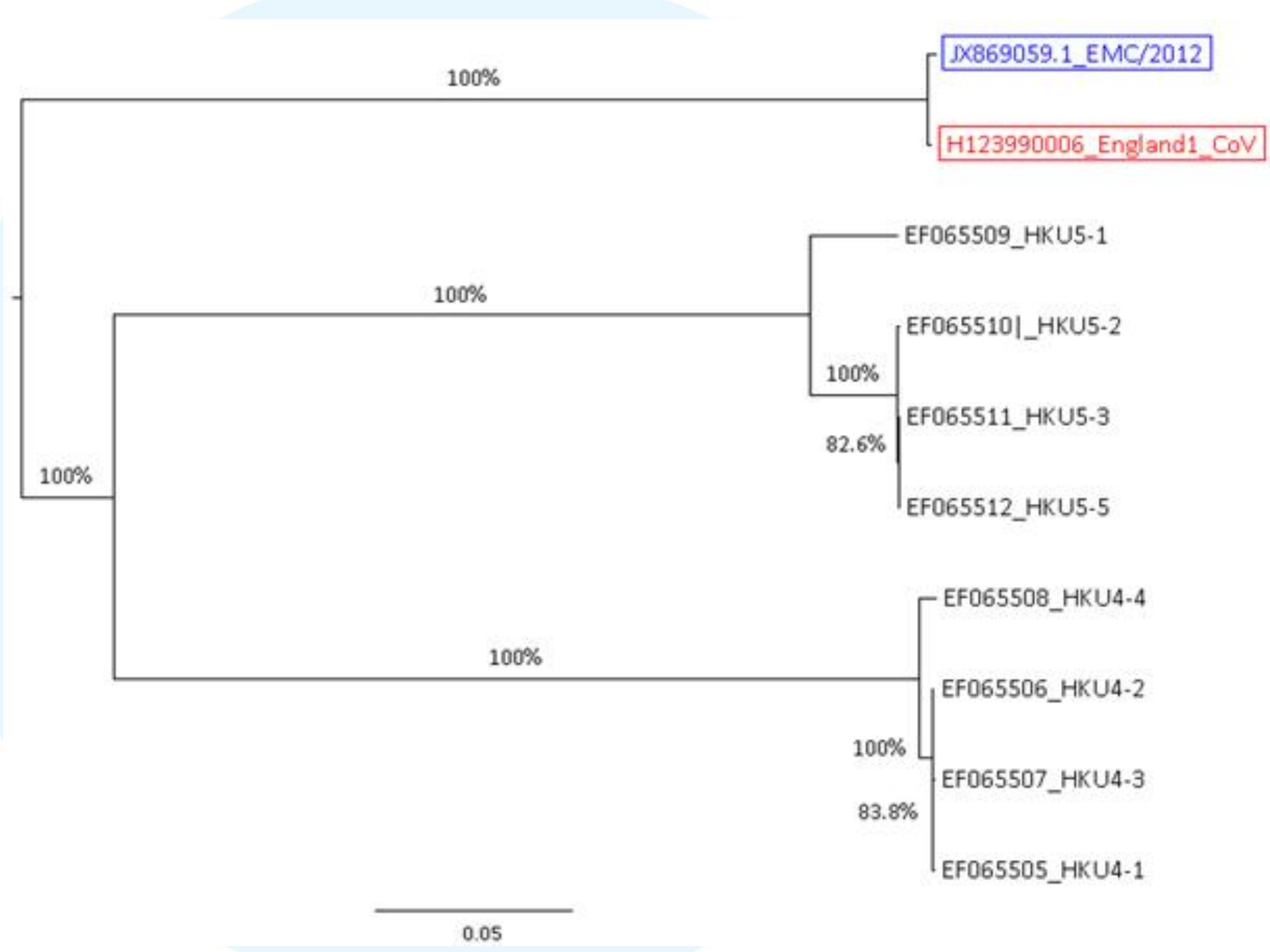
Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, van Boheemen S, Gopal R, Ballhause M, Bestebroer TM, Muth D, Müller MA, Drexler JF, Zambon M, Osterhaus AD, Fouchier RM, Drosten C. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39):pii=20285. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20285

Article submitted on 27 September 2012 / published on 27 September 2012

Diagnostic strategy – Subsequent

- Real-time reverse-transcription polymerase chain reaction
  - UpE
  - Orf1b
- Considerations
  - Positive control
  - Specimen type
  - Timing of specimen

13 November 2012


JX869059.1_EMC/2012
H123990006_England1_CoV
EF065509_HKU5-1
EF065510|_HKU5-2
EF065511_HKU5-3
EF065512_HKU5-5
EF065508_HKU4-4
EF065506_HKU4-2
EF065507_HKU4-3
EF065505_HKU4-1
100%
100%
100%
100%
82.6%
100%
100%
83.8%
0.05

Eurosurveillance, Volume 17, Issue 49, 06 December 2012

Rapid communications

ASSAYS FOR LABORATORY CONFIRMATION OF NOVEL HUMAN CORONAVIRUS (HCOV-EMC) INFECTIONS

V M Corman[1,2], M A Müller[1,2], U Costabel[3], J Timm[4], T Binger[1], B Meyer[1], P Kreher[5], E Lattwein[6], M Eschbach-Bludau[1], A Nitsche[5], T Bleicker[1], O Landt[7], B Schweiger[5], J F Drexler[1], A D Osterhaus[8], B L Haagmans[8], U Dittmer[4], F Bonin[3], T Wolff[5], C Drosten (drosten@virology-bonn.de)[1]

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Date of submission: 05 December 2012

We present a rigorously validated and highly sensitive confirmatory real-time RT-PCR assay (*1A* assay) that can be used in combination with the previously reported *upE* assay. Two additional RT-PCR assays for sequencing are described, targeting the *RdRp* gene (*RdRpSeq* assay) and *N* gene (*NSeq* assay), where an insertion/deletion polymorphism might exist among different hCoV-EMC strains. Finally, a simplified and biologically safe protocol for detection of antibody response by immunofluorescence microscopy was developed using convalescent patient serum.

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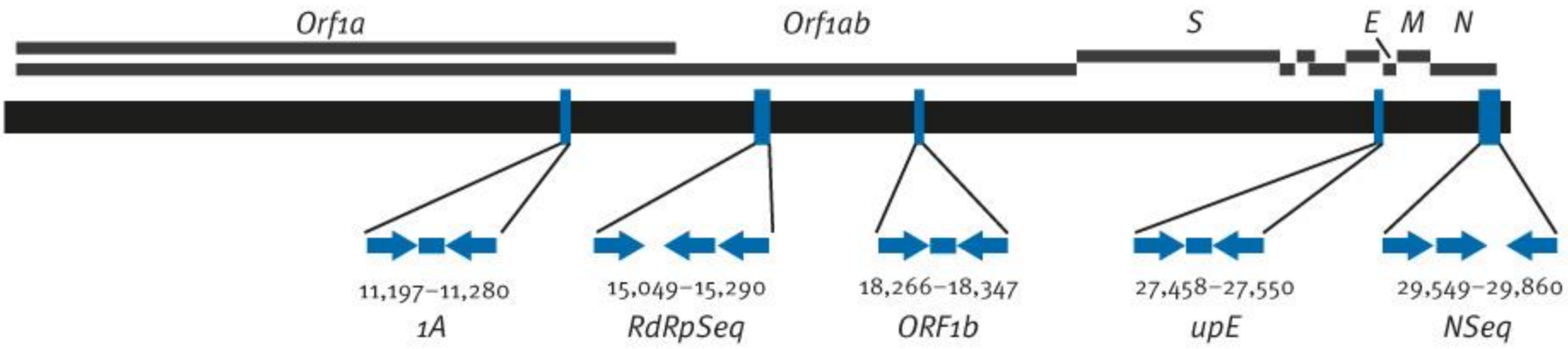
Laboratory capability for molecular detection and confirmation of novel coronavirus in Europe, November 2012
Ongoing outbreak of dengue type 1 in the Autonomous Region of Madeira, Portugal: preliminary report
Use of a geographic information system to map cases of measles in real-time during an outbreak in Dublin, Ireland, 2011
The application of geographic information systems and spatial data during Legionnaires' disease outbreak responses
Authors' correction for Euro Surveill. 2012;17(26)

Related articles

Laboratory capability for molecular detection and confirmation of novel coronavirus in Europe, November 2012
Incubation period as part of the case definition of severe respiratory illness caused by a novel coronavirus
The United Kingdom public health response to an imported laboratory confirmed case of a novel coronavirus in September 2012
Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012
Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction

FIGURE 1

RT-PCR target regions for screening, confirmation and sequencing of novel human coronavirus (hCoV-EMC)



N: nucleocapsid; *Orf*: open reading frame; *RdRp*: RNA-dependent RNA polymerase; RT-PCR: reverse transcription-polymerase chain reaction.

The figure shows the relative positions of amplicon targets presented in this study, as well as in [2]. Primers are represented by arrows, probes as blue bars. Numbers below amplicon symbols are genome positions according to the hCoV-EMC/2012 prototype genome presented in [1].

The *1A* assay is the confirmatory real-time RT-PCR test presented in this study (target in the *ORF1a* gene). The *RdRpSeq* assay is a hemi-nested sequencing amplicon presented in this study (target in the *RdRp* gene). The *ORF1b* assay is a confirmatory real-time RT-PCR presented in [2]. The *upE* assay is a real-time RT-PCR assay recommended for first-line screening as presented in [2] (target upstrem of *E* gene). The *NSeq* assay is a hemi-nested sequencing amplicon presented in this study (target in *N* gene).

Diagnostic tests

- Real-time reverse-transcription PCR
  - UpE
  - Orf1b
  - Orf1a
- Sequencing targets (conventional hemi-nested PCR)
  - RdRp (Orf1b) – Not for screening; primers cross-react with other coronaviruses
  - N (6 nt deletion in London strain) – Specific for novel virus; possibly for strain classification

Other tests

- Virus isolation
  - Technically feasible
  - Biosafety considerations
  - Positive isolate as early as 2 days after inoculation
  - Confirmation using PCR

Other tests

- Antibody detection
  - Biosafety considerations
  - Immunofluorescence (Western blot, neutralization)
  - Spike and nucleocapsid antibodies
  - For cases with no respiratory specimens within the first 10 days after onset
  - Paired acute and convalescent titres
  - Possibility of cross-reactivity

Indications for testing

- Clinical suspicion: Severe respiratory disease; renal failure
- Epidemiological consideration: Travel history; contact history?
- Obtain specimens based on disease course
- Liaise with laboratory as necessary

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Thank you

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