MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS

MERS-COV

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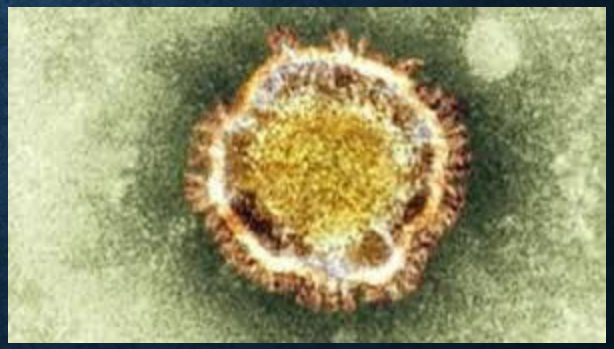
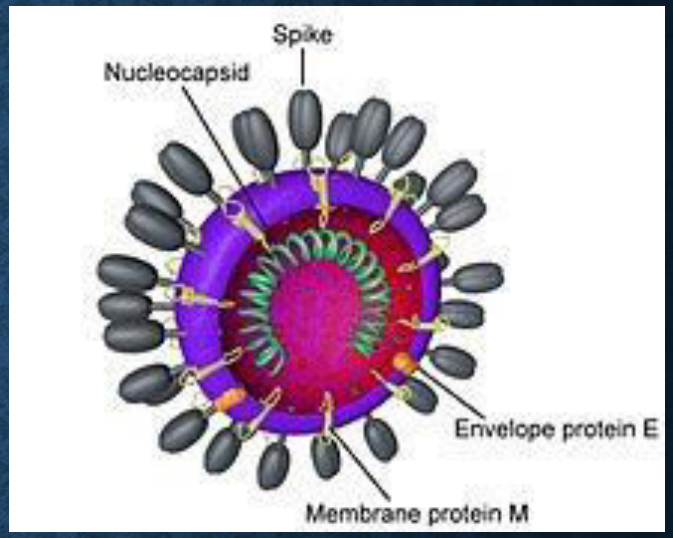
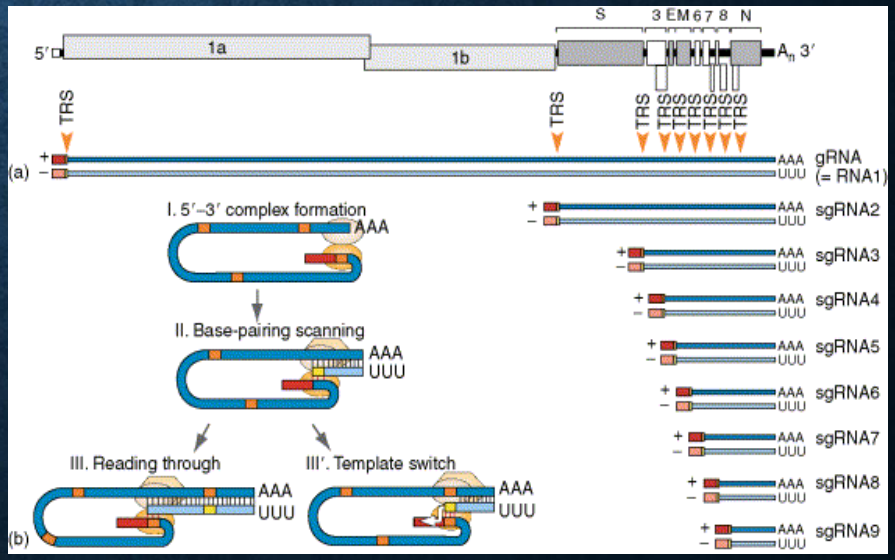
VRC, NRITLD
11 Dec 2014



CORONAVIRUSES (COVS)

- infect and cause disease in a wide variety of species
  - bats, birds, cats, dogs, pigs, mice, horses, whales, and humans
  - Until 2003, HCoV-229E and HCoV-OC43
  - In 2002 to 2003, SARSCoV
  - 2004 ,HCoV-NL63
  - 2005 , HCoV-HKU1
- large, enveloped, positive-stranded RNA viruses, large RNA genomes
  - replicate by a similar and unique mechanism
    - high frequency of recombination
    - a 3′-coterminal, nested-set structure of the mRNAs

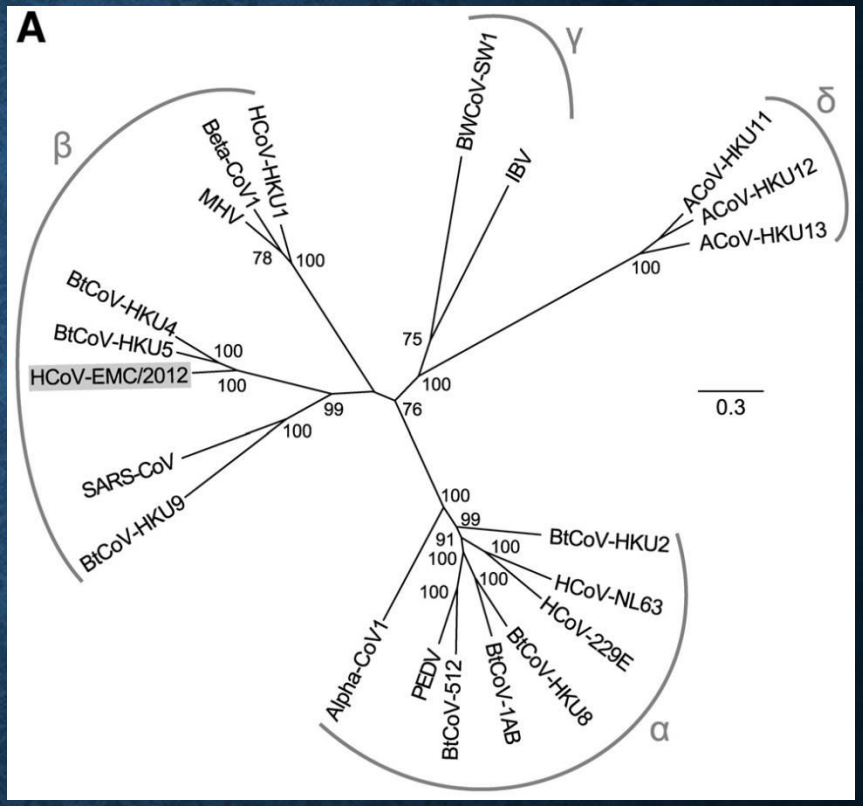




CLASSIFICATION

- *Coronavirinae subfamily:*
  - *Alphacoronavirus*
  - *Betacoronavirus*
  - *Gammacoronavirus*
  - *Deltacoronavirus*
    - ✓ **genus α; HCoV-229E / HCoV-NL63**
    - ✓ **genus β ; OC43 / HKU1 / SARS-CoV**
    - ✓ **genus γ & δ; birds**
- **"rule"; Most coronaviruses infect only one animal species or, at most, a limited number of closely related species.**
  - **SARS-CoV; an exception to this "rule" to infect a wide range of mammals**

    humans, nonhuman primates, Himalayan palm civets, raccoon dogs, cats, dogs, and rodents

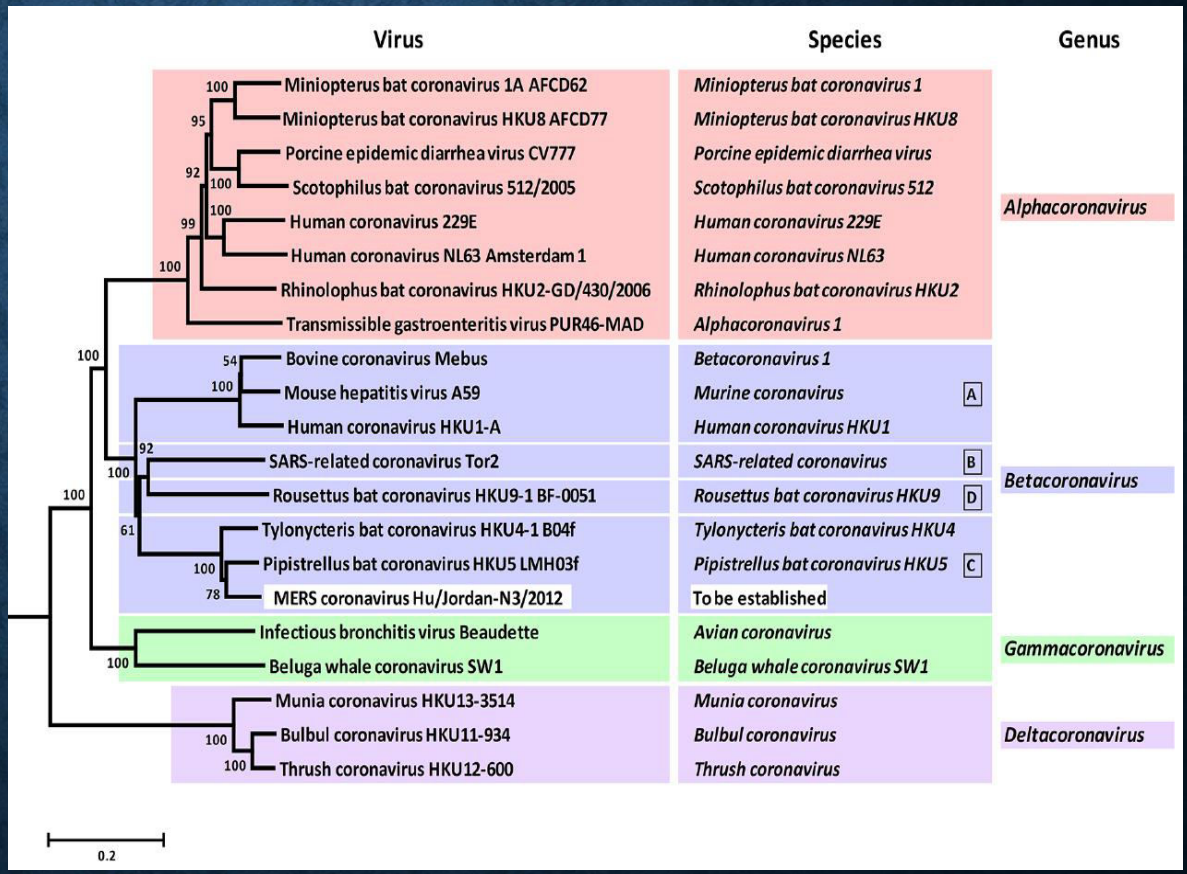


•SARS-CoV diversity based on the nucleocapsid (N) gene

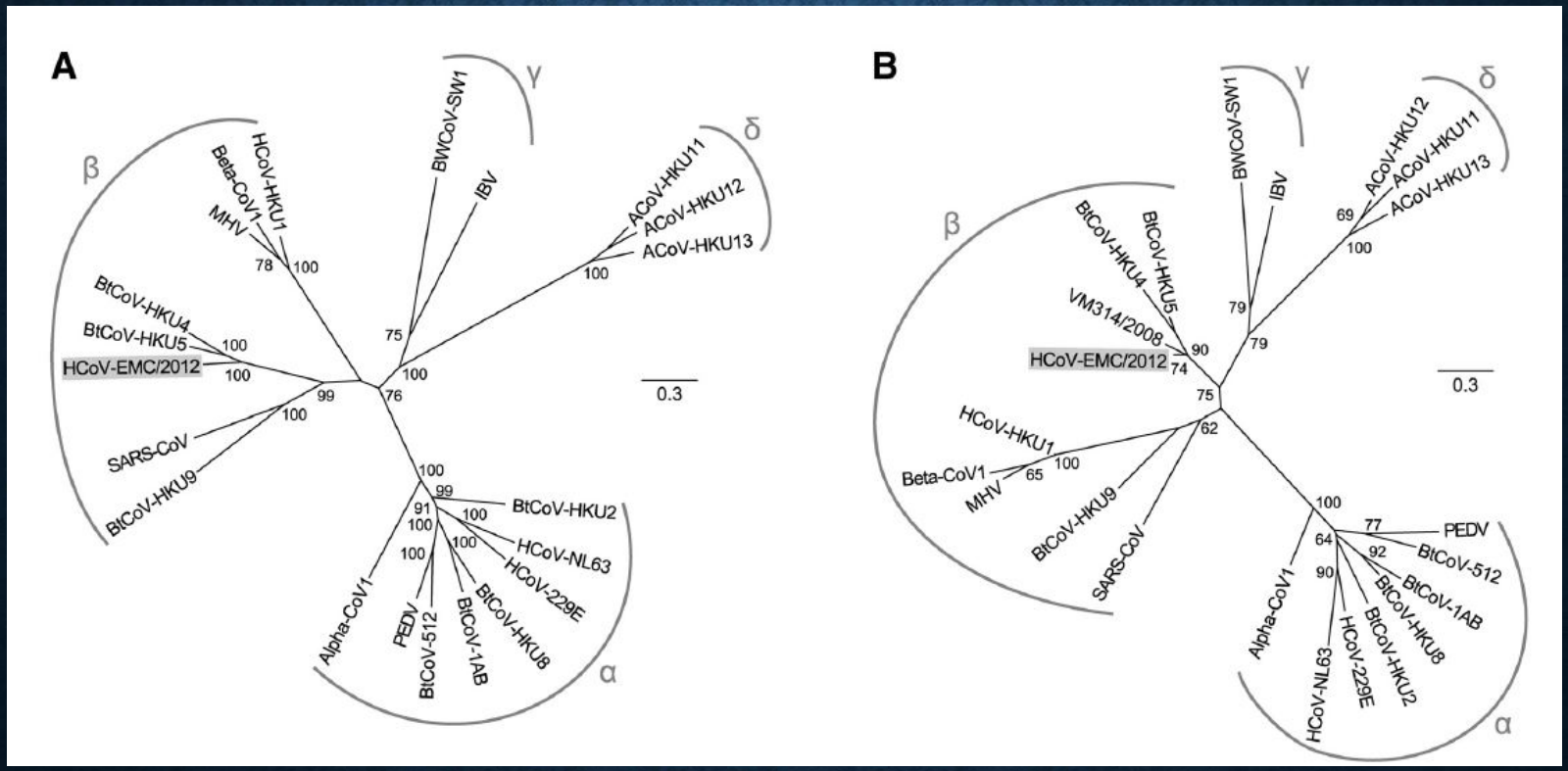
- **Several SARS-like CoVs been detected in bats; 3% to 6% nt diversity with SARS-CoV**
- **The group of SARS-CoVs detected in humans and animals in wild animal markets in 2003 and 2004 have ≤0.5% nt diversity in the N gene.**

MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS MERS-COV

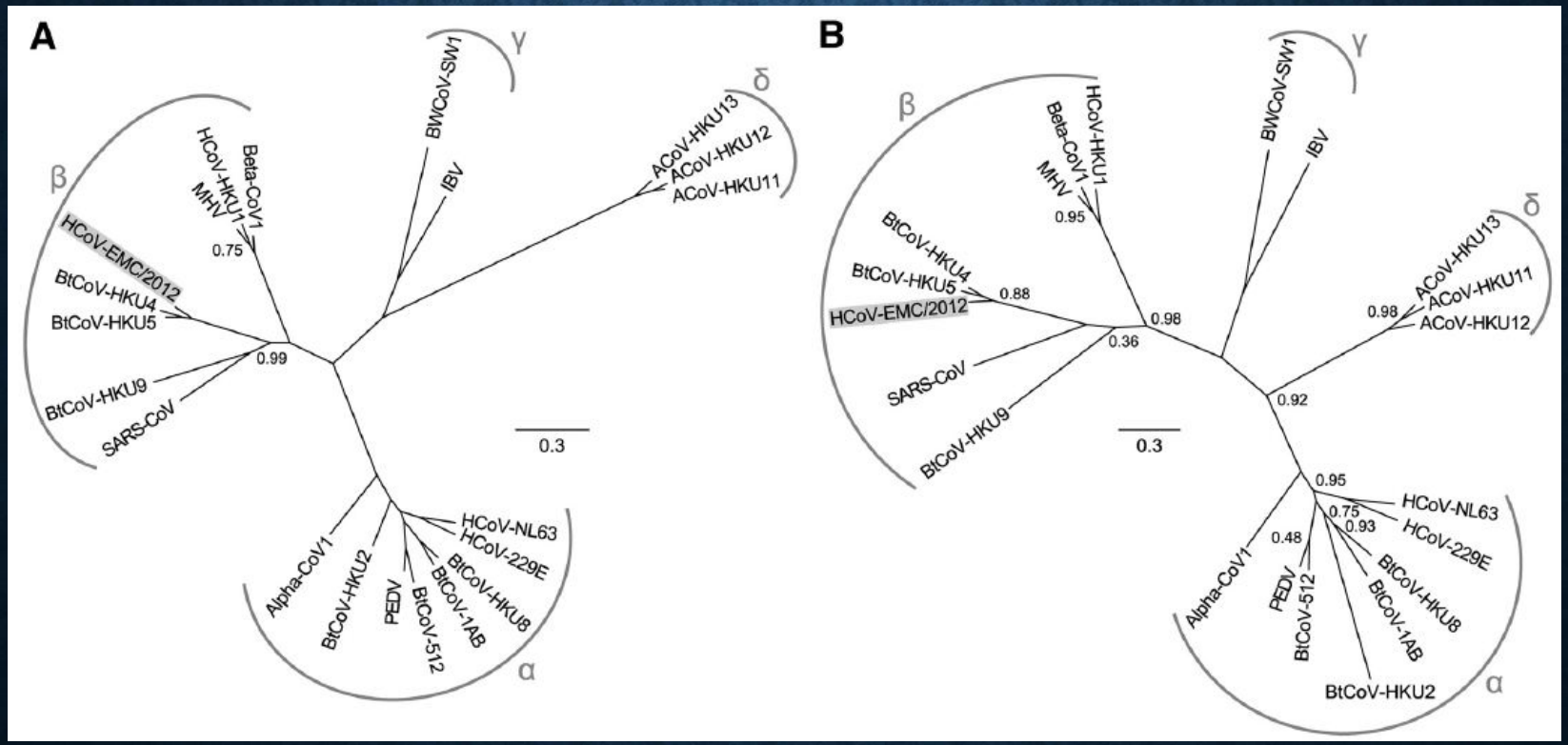
- **firstly; HCoV-EMC; "Erasmus Medical Center" , the place where the virus characterized in Netherland.**
  - **isolated from the sputum of a 60-year-old, Saudi Arabia**
  - **Viral Genome Sequencing of pan-coronavirus PCR amplicons**
    - **HCoV-EMC belonged to the lineage C of genus betacoronavirus; 1ST HCoV in lineage C**
    - **HCoV-HKU1 / HCoVOC43; lineage A**
    - **SARS-CoV; lineage B**
    - **Lineage D does not contain any human pathogens**



PHYLOGENETIC TREES FOR HCOV-EMC/2012 AND SELECTED OTHER CORONAVIRUSES. UNROOTED MAXIMUM LIKELIHOOD PHYLOGENIES INFERRED FROM THE NUCLEOTIDE SEQUENCES OF FULL-LENGTH ORF1AB (A) OR A 332-NT FRAGMENT FROM THE RDRP-ENCODING DOMAIN OF ORF1B (B)



PHYLOGENIES BASED ON CORONAVIRUS-WIDE CONSERVED PROTEIN DOMAINS IN REPLICASE PP1AB (A) OR ON THE CONSERVED PARTS OF STRUCTURAL PROTEINS S2, E, M, AND N (B)



COUNTRIES WITH LAB-CONFIRMED MERS CASES

Countries in or near the Arabian Peninsula with Cases

- Saudi Arabia
- United Arab Emirates (UAE)
- Qatar
- Oman
- Jordan
- Kuwait
- Yemen
- Lebanon
- Iran

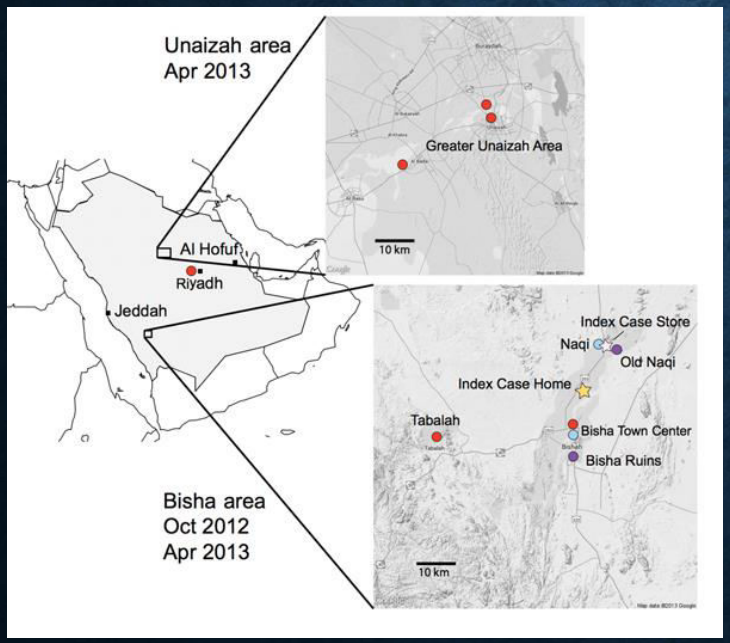
Two patients were transferred to Germany for care.

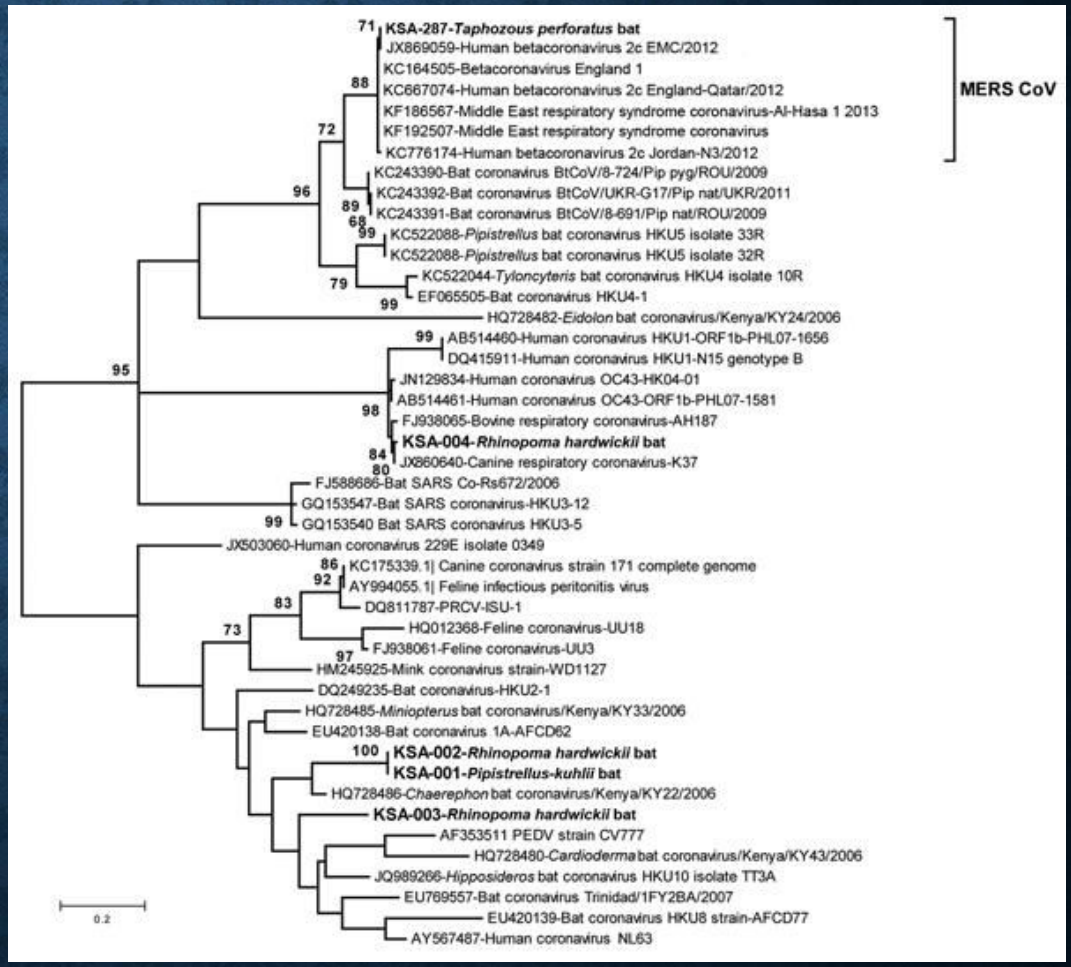
Countries with Travel-associated Cases

- **United Kingdom (UK)**
- **France**
- **Tunisia**
- **Italy**
- **Malaysia**
- **Philippines**
- **Greece**
- **Egypt**
- **United States of America (USA)**
- **Netherlands**
- **Algeria**
- **Austria**
- **Turkey**

MERS VIRUS DISCOVERED IN BAT NEAR SITE OF OUTBREAK IN SAUDI ARABIA

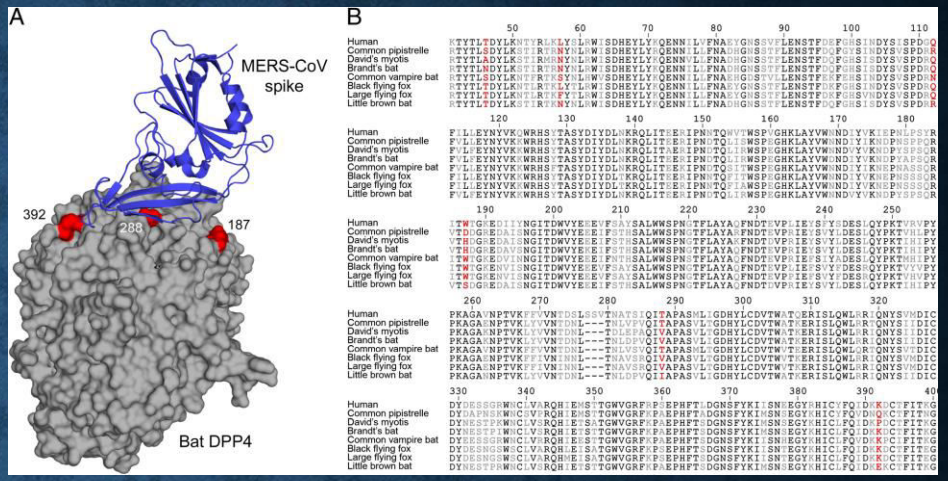
- Molecular investigation indicated that bats in Saudi Arabia are infected
- with several alphacoronaviruses and betacoronaviruses.
- Virus from 1 bat showed 100% nucleotide identity to virus from the human index case-patient.
- Bats might play a role in human infection.

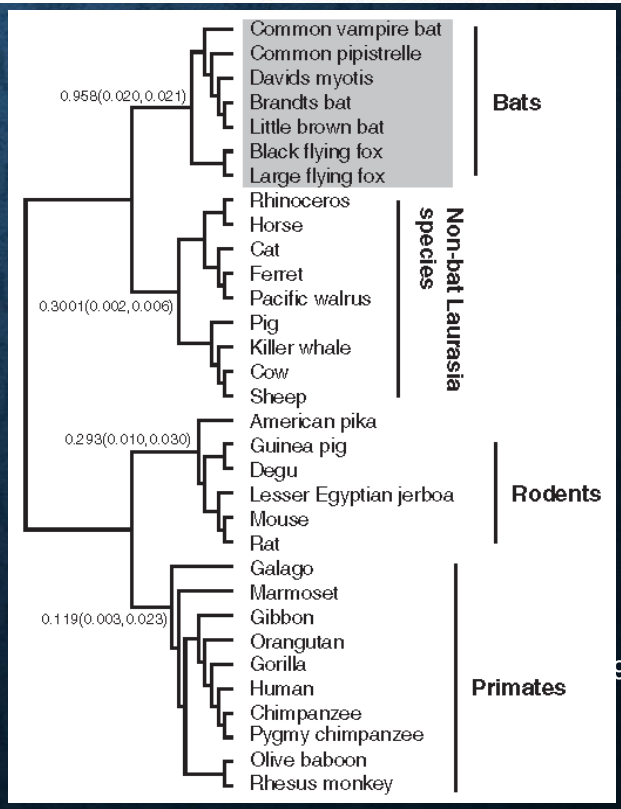




ORIGIN OF MERS CORONAVIRUS

- newly emerged MERSCoV not only has a bat origin, but also evolved over an extended time period in bat populations before making the leap to infect humans
  - Bat DPP4 genes have adapted significantly as they evolved, suggesting a long-term arms race between the bat and the virus.
- Evolutionary footprints in receptor-encoding genes of hosts and their binding domains during long battles with the hosts
  - jumping species boundaries to infect humans, perhaps through an intermediate host





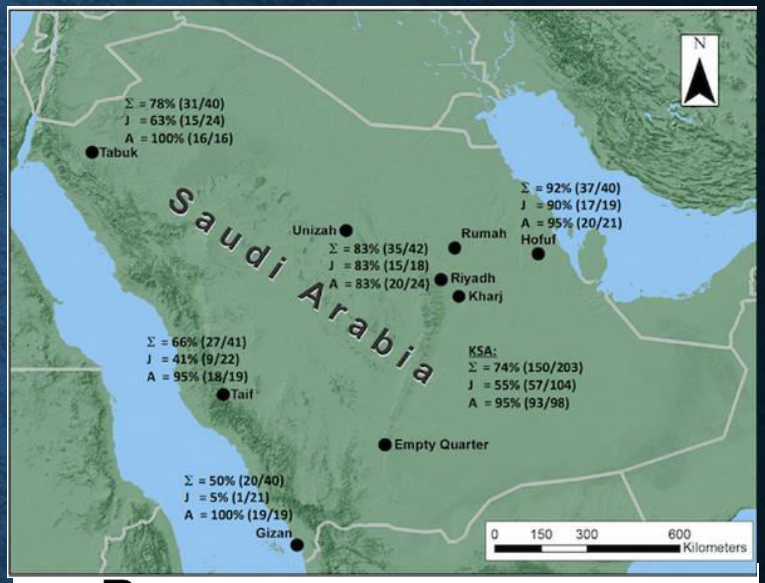
MERS CORONAVIRUS CAN BE TRANSMITTED FROM CAMEL TO HUMANS

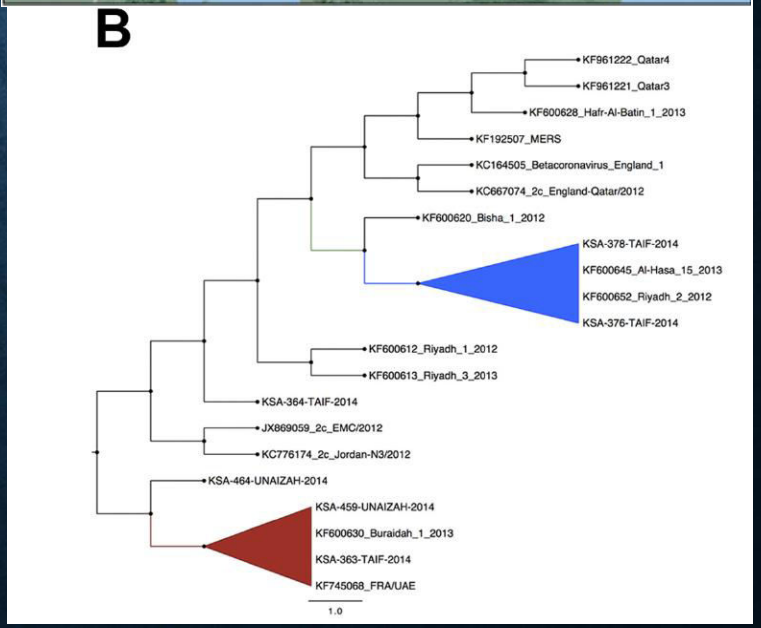
- **Viruses in humans and camels from one region are identical**
  - **This indicates transmission between animals and man**
  - **Virus RNA differs from region to region**
- **Transmission pathway through nose and eyes**
  - **Virus levels high in the nasal mucosa and conjunctiva of camels**
  - **Transmission through these contact sites, especially through nasal discharge**
- **MERS and SARS coronaviruses are relatives**
  - **SARS coronavirus probably passing from bats to humans**
  - **MERS coronavirus is being constantly transmitted from camels to humans**

MERS coronavirus is transmitted not only between animals but also from camels to humans.

MERS VIRUS WIDESPREAD IN SAUDI ARABIAN CAMELS: CORONAVIRUS HAS BEEN INFECTING THE ANIMALS FOR AT LEAST 20 YEARS

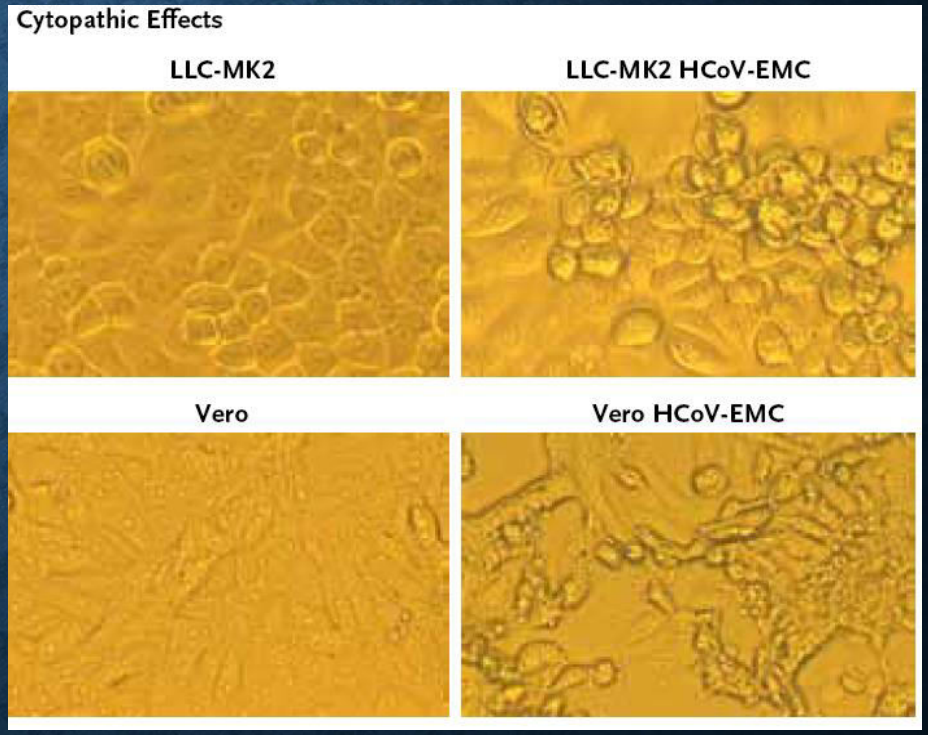
- **Blood samples from dromedary camels taken from 1992 through 2010**
  - **infection in camels typically occurs in early life**
    - **Overall, 74% of camels sampled**
    - **Adult camels were more likely to have antibodies to the virus (>80%)**
    - **juveniles were more likely to have active virus.**
      - **age two or younger the prevalence ranged from 90% in the east to 5% in the southwest**
      - **people get the virus from camels the most likely source is young camels**
- **Active virus in nasal swabs in 35% of young camels and 15% of adult camels countrywide**
  - **Less frequently found in rectal swabs and not in blood**
  - **virus most likely is spread by respiratory secretions**





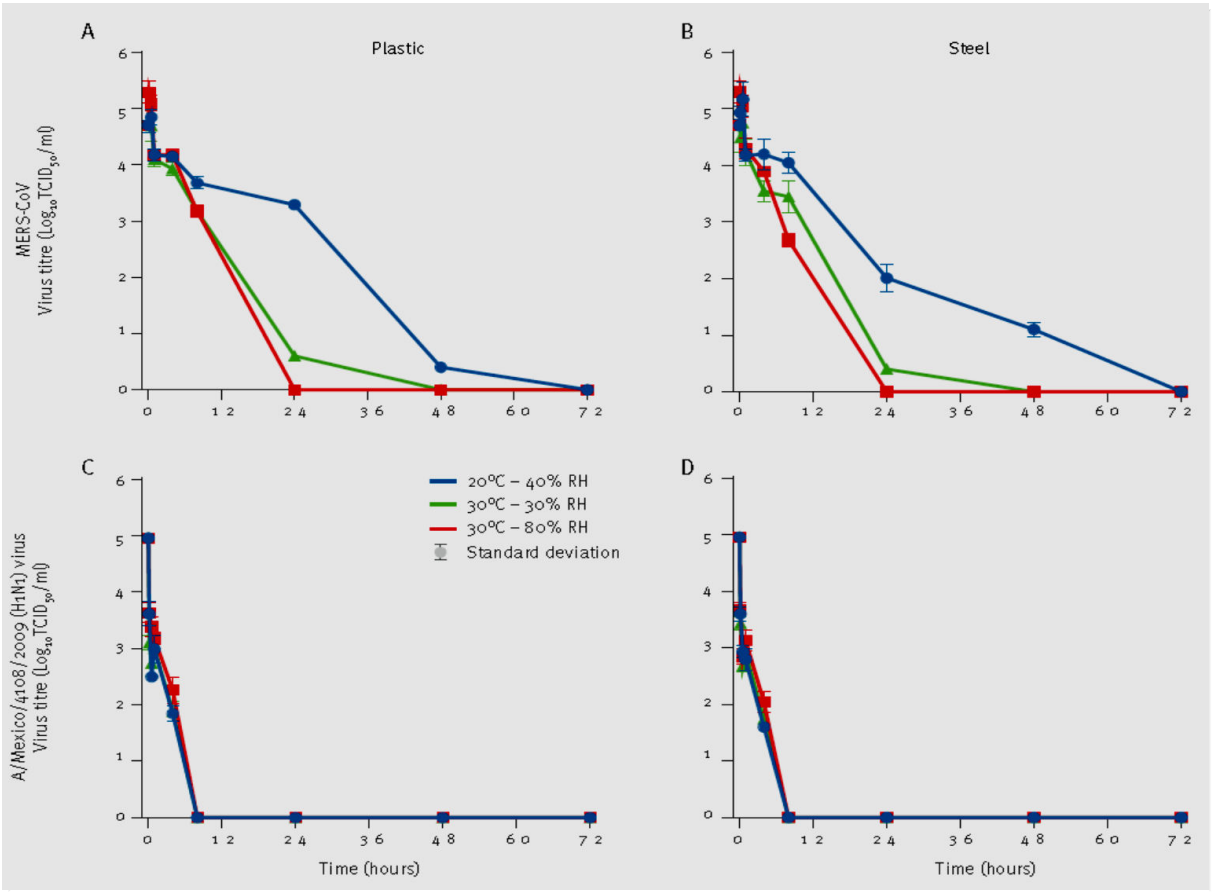
OTHER CHARACTERISTIC OF MERS-COV

- **MERS-CoV** was isolated and propagated relatively easily in Vero B4 and LLC-MK2 cells
  - **SARS-CoV and HCoV-NL63**
  - **Cell Culture: In certain circumstances, but not routine diagnosis, laboratories with the appropriate experience and containment facilities, may attempt to isolate the virus in cell culture.**



STABILITY OF MERS-COV IN ENVIRONMENT

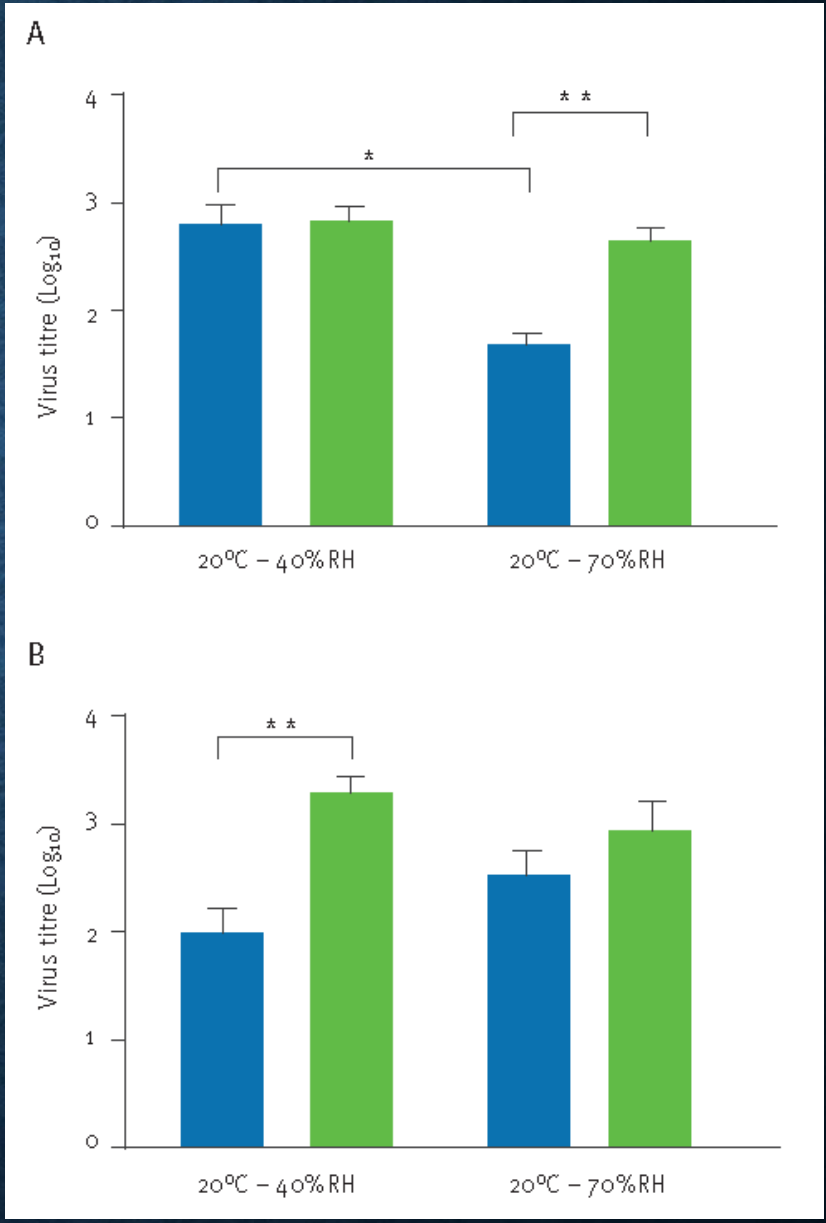
compared to A(H1N1)09pdm MERS-CoV remains viable for a longer duration in the environment



After four hours no viable A(H1N1)09pdm virus was detected in comparison to 8, 24 or 48 hours for MERS-CoV depending on environmental conditions

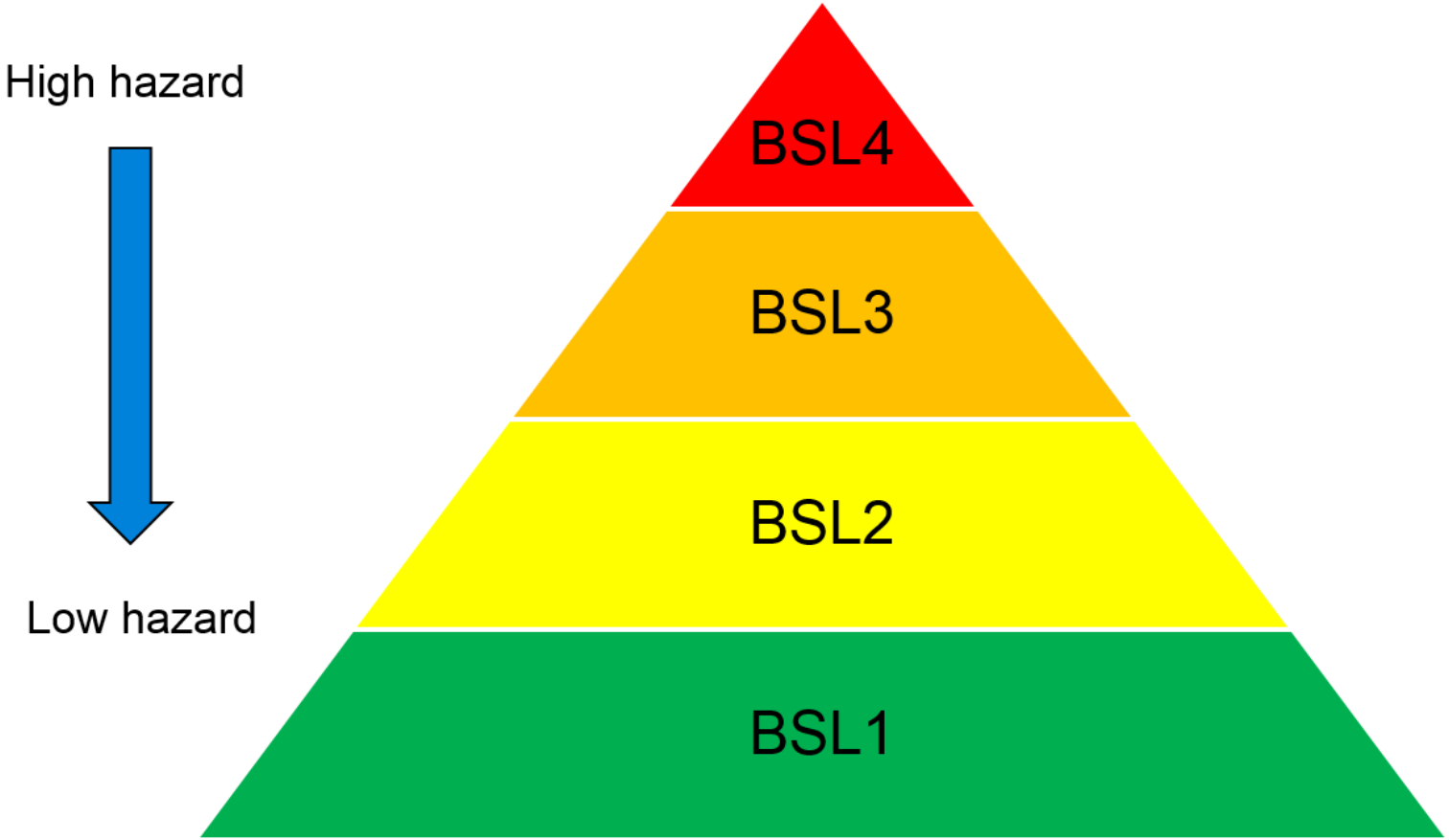
STABILITY OF MERS-COV IN ENVIRONMENT

- MERS-CoV was very stable in aerosol form at 20°C – 40% RH.
- The decrease in viability at 20°C – 70% RH (89%) was comparable to that of A(H1N1)09pdm
- MERS-CoV and SARS-CoV share relatively similar stability characteristics
  - SARS-CoV has been reported to stay viable for up to five days at 22 to 25°C and 40 to 50% RH and increase in temperature and humidity resulted in a rapid loss of viability
- The prolonged survival of MERS-CoV compared to A(H1N1)09pdm on surfaces increases the likelihood of contact and fomite transmission.
  - decrease in viability observed at high temperature suggests that direct contact transmission, and not fomite transmission, in the Arabian Peninsula would be the most likely route of zoonotic and human to human transmission in outdoor settings



LABORATORY CLASSIFICATION

Containment levels for work will depend on potential exposure to different groups of biological agents.



LABORATORY BIORISK MANAGEMENT FOR LABORATORIES

- WHO recommends that all diagnostic laboratory work and PCR analysis on clinical specimens taken from patients who are suspected or confirmed to be infected with MERS coronavirus should be conducted according to practices and procedures described for basic laboratory — Biosafety Level 2 (BSL2)

- Routine laboratory procedures that require BSL-2 include:
  - ✓ Diagnostic testing of serum, blood (including haematology and clinical chemistry), respiratory tract specimens, or other specimens;
  - ✓ Manipulations involving neutralized or inactivated (lysed, fixed, or otherwise treated) virus particles and/or incomplete, non-infectious portions of the viral genome.
    - ✓ The reason for working with these types of specimens in a BSL-2 facility is to protect the specimen from contamination and accordingly the quality of the specimen;
  - ✓ Routine examination of mycotic and bacterial cultures developed from respiratory tract specimens.

BSL2

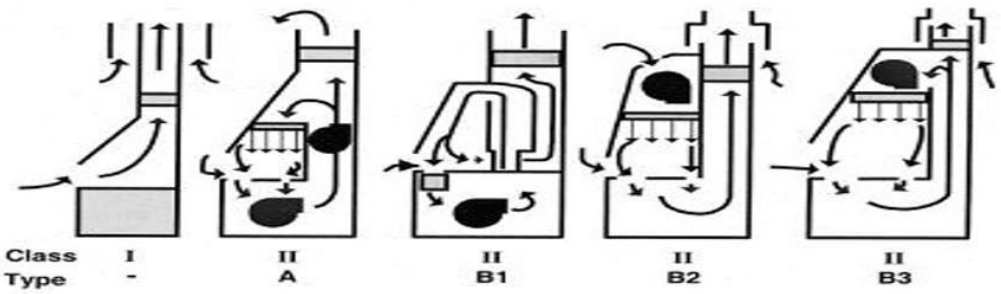
- PROHIBITED; Eating, drinking, smoking, applying cosmetics, and handling contact lenses in the laboratory working areas.
- **Personal Protective Equipment (PPE)**
  - masks, respirators, lab coats, etc.
- All manipulations of potentially infectious materials should be performed in appropriately maintained and validated biological safety cabinets
  - Use of Class II BSCs should be considered to protect work surface materials as well as personnel and the environment.
- **Appropriate disinfectants**
- **Contaminated waste**

CLASS II BSC

The Class II BSC was designed not only to provide personnel protection but also to protect work surface materials from contaminated room air.

Class II BSCs, of which there are four types:

- Class II type A1
- Class II type A2
- Class II type B1
- Class II type B2



BSC	FACE VELOCITY (m/s)	AIRFLOW (%)		EXHAUST SYSTEM
		RECIRCULATED	EXHAUSTED	
Class I[a]	0.36	0	100	Hard duct
Class IIA1	0.38–0.51	70	30	Exhaust to room or thimble connection
Class IIA2 vented to the outside[a]	0.51	70	30	Exhaust to room or thimble connection
Class IIB1[a]	0.51	30	70	Hard duct
Class IIB2[a]	0.51	0	100	Hard duct

SPECIMEN COLLECTION AND SHIPMENT

- Lower respiratory tract specimens ; the highest virus titer
  - tracheal aspirates and BAL
- Upper respiratory tract specimens recommended
  - especially when lower respiratory tract specimens cannot be collected
- Paired serum samples pending the availability of serological assays
  - collected at least 21 days apart, with the first being collected during the first week of illness
- Specimens testing for the novel coronavirus should preferably be tested to exclude the presence of known respiratory pathogens.
- Specimens
  - should reach the laboratory as soon as possible after collection.
  - The importance of proper handling during transportation
    - When there is likely to be a delay in the laboratory receiving respiratory tract specimens or serum, it is strongly advised to freeze them on dry ice.

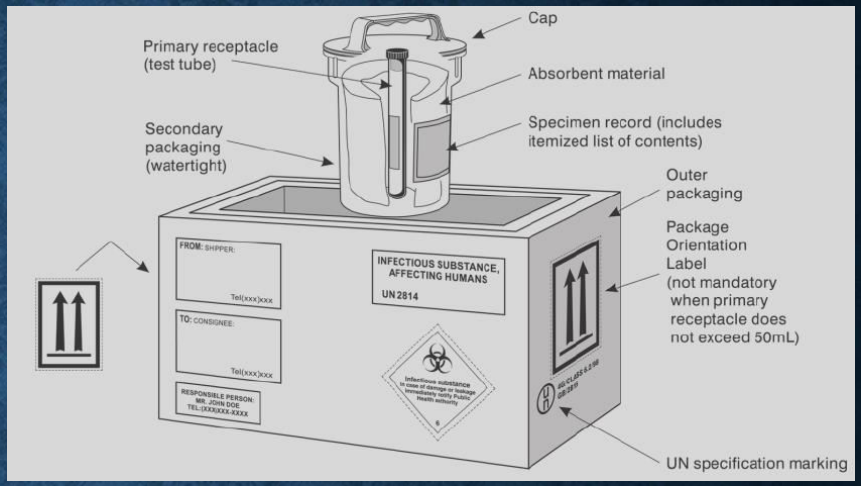
Types of specimens for testing for the presence of MERS-CoV

Specimen type	Transport medium	Transport to laboratory	Dangerous goods shipping category	Comment
Naturally produced sputum*	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	Biological substance, Category B	Need to ensure the material is from the lower respiratory tract
Bronchoalveolar lavage	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	There may be some dilution of virus but still a worthwhile specimen
Tracheal aspirate	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Nasopharyngeal aspirate	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Combined nose/throat swab	Virus transport medium	On ice.	As above	Virus has been detected in this type of specimen
Nasopharyngeal swab	Virus transport medium	On ice.	As above	
Tissue from biopsy or autopsy including from lung	Virus transport medium or saline	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Serum for serology or virus detection: always collect paired samples if possible. Acute – first week of illness	no	On ice **or** frozen	As above	
Convalescent - ideally 3 to 4 weeks later				
Whole blood	EDTA anticoagulant	On ice	As above	For virus detection, particularly in the first week of illness

INFECTIOUS SUBSTANCES IN CATEGORY A

- **Category A**
  - *An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.*
  - *UN 2814; cause disease in humans or both in humans and animals*
  - *UN 2900 ; cause disease only in animals*

"suspected category A infectious substance"



SHIPPER'S DECLARATION FOR DANGEROUS GOODS

Shipper
Dr XY Orange, tel 0789 456 123
Childrens' hospital
4, Splendid Street
12345 Beautiscity
Nicecountry

Air Waybill No. 543 7654 9876
Page 1 of 1 Pages
Shipper's Reference Number (optional)

Consignee
Dr AB Normal, tel 03219 987 4568
Virobactfung Laboratories
6, Many Way
98765 Mydface
Hercountry

Two completed and signed copies of this declaration must be handed to the operator.

TRANSPORT DETAILS

This shipment is within the limitations prescribed for: *(Delete non-applicable)*
PASSENGER AND CARGO AIRCRAFT / CARGO AIRCRAFT ONLY

Airport of Departure: Amleaving

Airport of Destination: Willgetthere

WARNING
Failure to comply in all aspects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties.

Shipment Type *(Delete non-applicable)*
NON-RADIOACTIVE / RADIOACTIVE

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods Identification

UN or ID No.	Proper Shipping Name	Class or Division (Subsidiary Risk)	Packing Group	Quantity and Type of Packing	Packing Inst.	Authorization
UN 2014	Infectious substance, affecting humans (Ebola virus)	6.2		50ml	620	
UN 1845	Dry ice	9		20kg All packed in one fibreboard box	954	

Additional Handling Information
Emergency contact: Dr Orange tel 0789 456 123

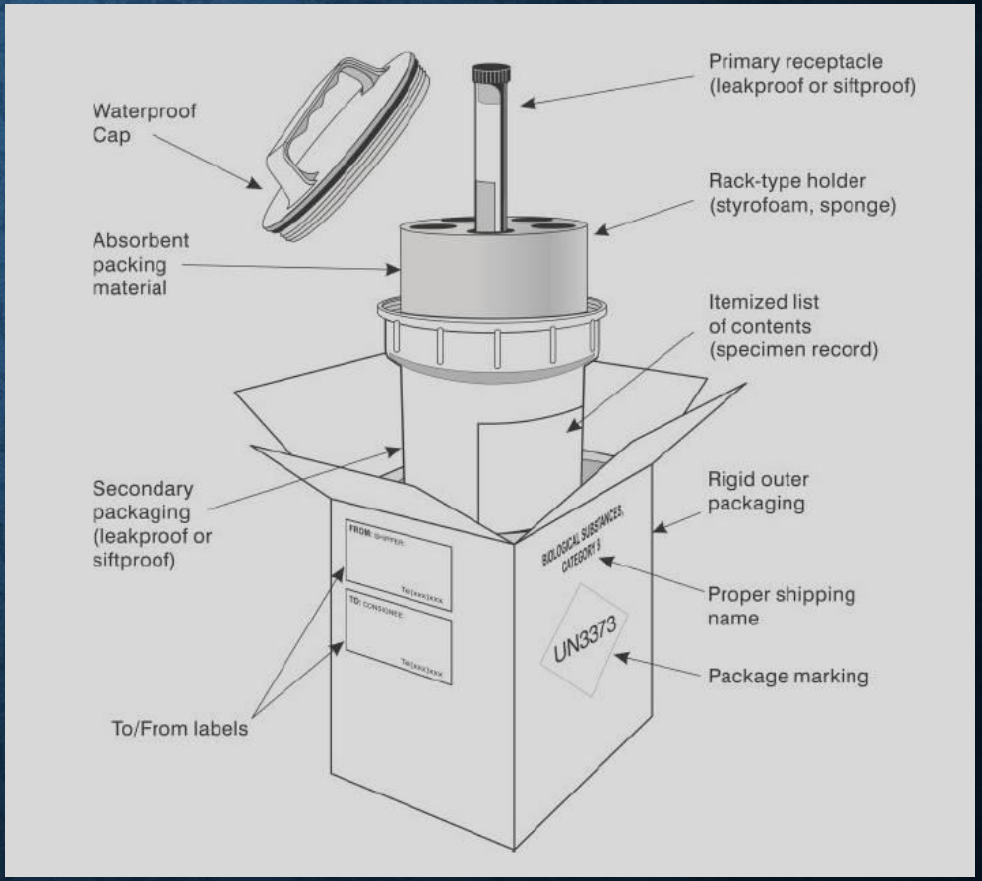
I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations.

Name/Title of Signatory: Dr XY Orange, Goods Dispatch
Place and Date: Beautiscity 18 June 2010
Signature *(see warning above)*

INFECTIOUS SUBSTANCES IN CATEGORY B

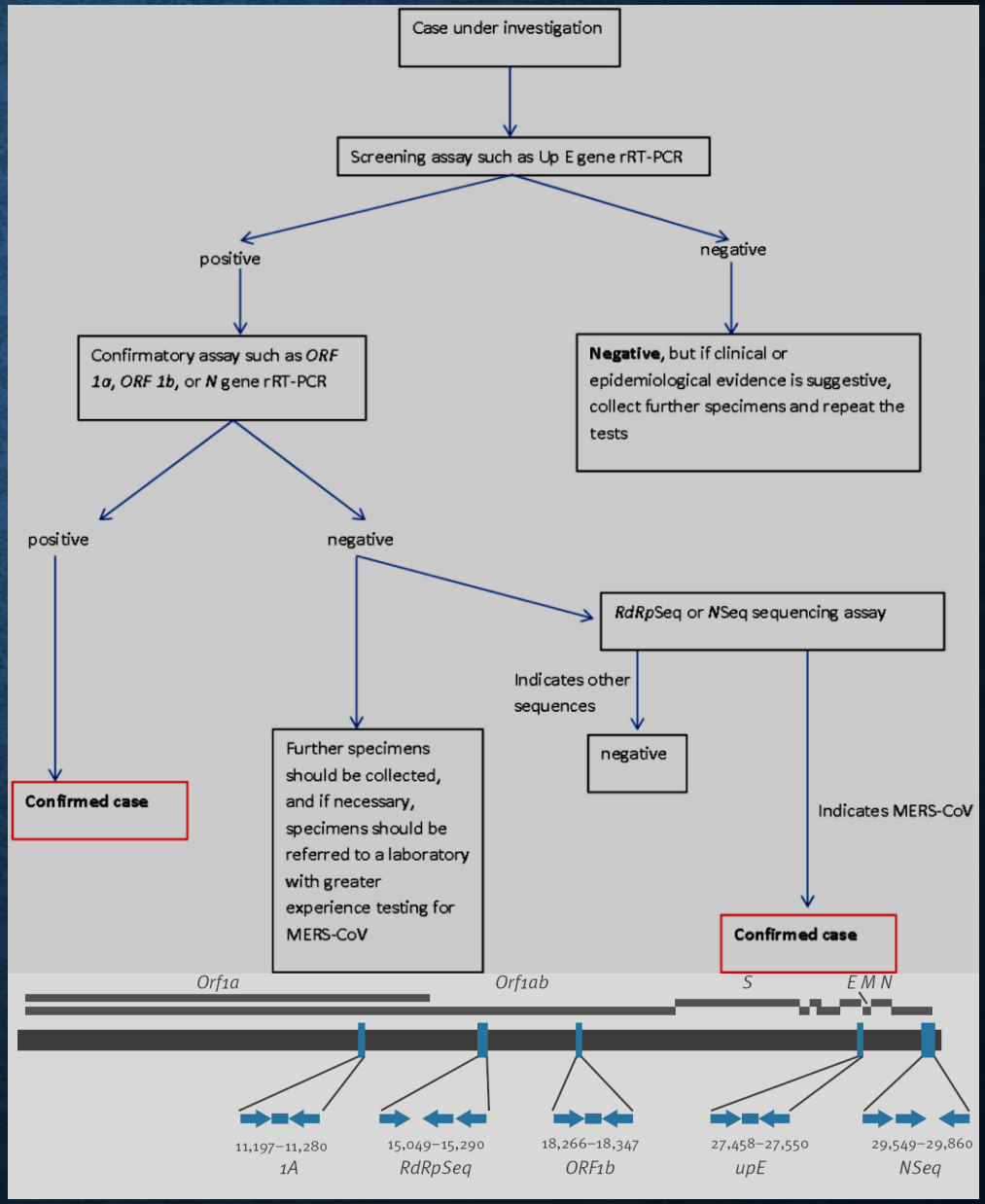
Category B

- *An infectious substance which does not meet the criteria for inclusion in Category A.*
- *Infectious substances in Category B shall be assigned to UN 3373.*
  - ***NOTE:*** ***The proper shipping name of UN 3373 is "BIOLOGICAL SUBSTANCE, CATEGORY B".***



LABORATORY TESTING METHODS AND ALGORITHM

- **Routine confirmation of cases; be based on detection of viral RNA by real-time RT-PCR and sequencing.**
  - **Detection: RT-PCR assays that are specific for the novel coronavirus**
    - ***Up-stream E protein gene (upE), ORF 1b gene and ORF 1a gene***
      - ***upE target ; highly sensitive,***
      - ***ORF 1a assay ; equal sensitivity***
      - ***ORF 1b assay ;***
        - ***less sensitive than the ORF 1a assay but may be more specific***
  - **Sequencing: sequencing to aid confirmation**
    - ***RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) protein genes***
      - **sequencing of an amplicon is useful for discordant results with two assays**
      - **provide valuable information to help understand the origins of the virus**
      - **sequencing of nucleic acid from as many positive specimens as possible is recommended**



Virology Research Center

RT-PCR false-negative results importance of serological testing

- A number of factors could result in false-negative results, including:
  - ✓ poor quality of specimen, such as a respiratory tract specimen containing primarily oropharyngeal material
  - ✓ the specimen was collected late or very early in the illness
  - ✓ the specimen was not handled and shipped appropriately
  - ✓ technical reasons inherent in the test, e.g., virus mutation or PCR inhibition
- When the clinical presentation and epidemiology suggest an infection with novel coronavirus despite negative PCR results, serological testing may be useful to confirm infection.
  - This highlights the importance of collecting paired serum samples from cases under investigation.

INTERPRETATION OF LABORATORY RESULTS

- positive PCR assays for at least two different specific targets on the novel coronavirus genome

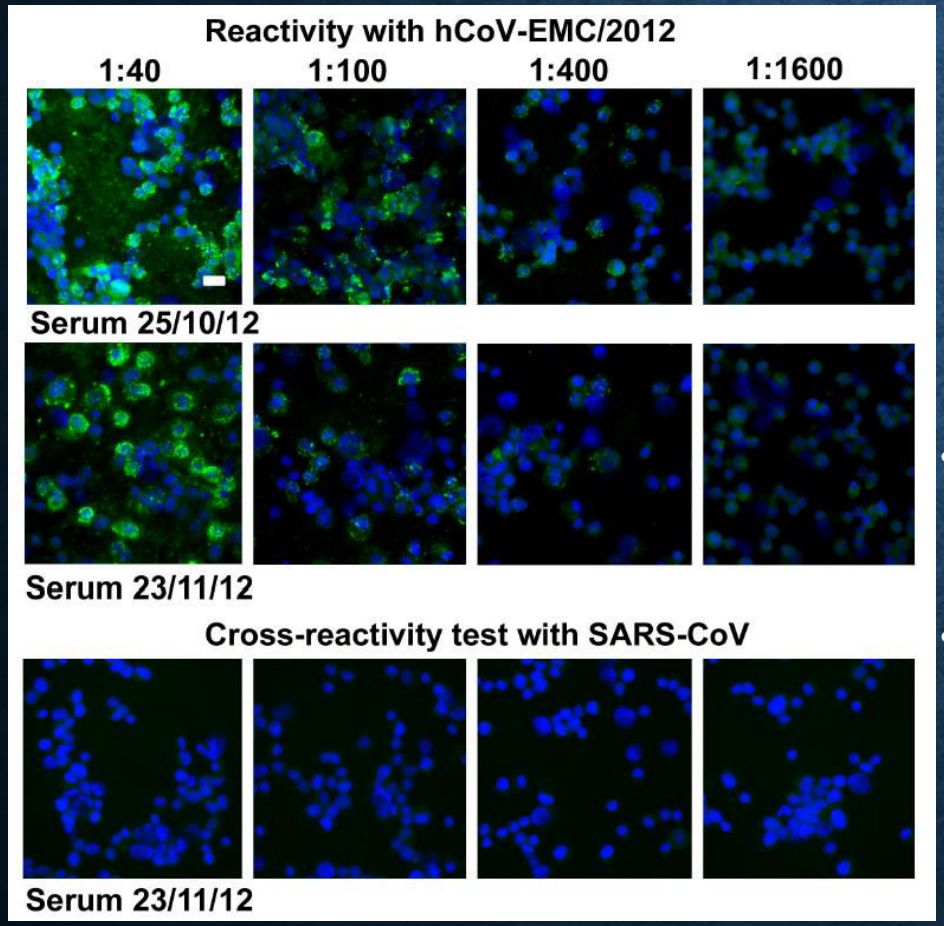
OR

- one positive PCR assay for a specific target on the novel coronavirus genome and an additional different PCR product sequenced, confirming identity to known sequences of the new virus

SEROLOGICAL TESTING RECOMMENDATIONS

- No official recommendations are currently available regarding serological
- Currently recommended a two-step diagnostic approach:
  - Step 1. Conventional immunofluorescence assay (cIFA)
    - Substrate (glass slides): MERS-CoV infected and uninfected Vero cells
    - Diagnostic parameters: Immunoglobulin G (Ig)G and IgM in patient serum
  - Step 2.
    - A) Recombinant IFA (rIFA) using spike protein
      - Substrate (glass slides): Transfected Vero cells expressing recombinant spike protein of MERS-CoV. Remark: For differential rIFA prototype humanpathogenic CoV (HCoV-NL63, -229E, -OC43, -HKU-1, SARS-CoV) should be included.
      - Diagnostic parameters: Immunoglobulin G (Ig)G and IgM in patient serum
    - B) Confirmation by virus plaque reduction neutralization test (PRNT)
      - Testing material: Heat-inactivated serum/ dilutions starting at 1:20 until 1:640
      - Diagnostic parameter: MERS-CoV neutralizing Ig in patient serum

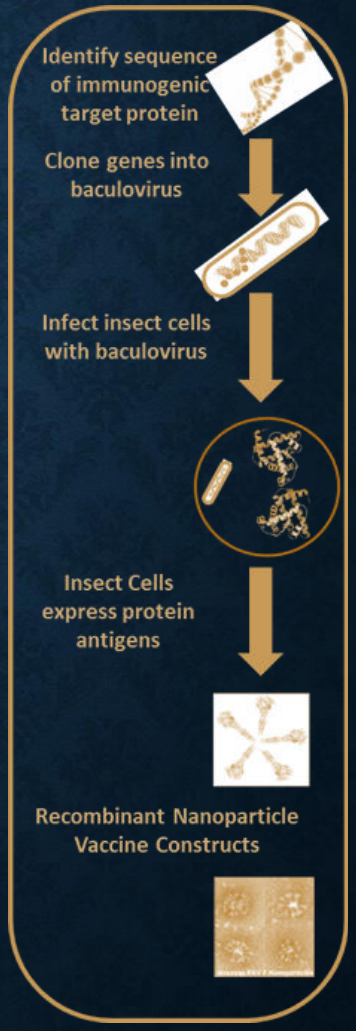
SEROLOGICAL TESTING



- The use of IFA is only to be applied in individuals with symptoms according to WHO case definition and a history of proven exposure to a laboratory-confirmed case of MERS-CoV during the infectious period.
  - **IFA seroconversions usually began to show from day 10 of symptoms onward**
    - **while virus RNA could not be detected by RT-PCR in respiratory secretions starting from day 15 onward**
- MERS-CoV provides a fine granular cytoplasmatic fluorescence pattern sparing the nucleus.
- IFA will generate false-positive results due to cross-reactivity between anti-HCoV antibodies. **such as OC43 and HKU1?**
  - impossible to discriminate cross-reactive antibodies by IFA

MERS-COV VACCINE CANDIDATE

- **Novavax** on June , 2013 announced that it had successfully produced a vaccine candidate designed to provide protection against the recently emerging MERS-CoV.
- The vaccine candidate was made using **Novavax** nanoparticle vaccine technology, is based on the major surface spike (S) protein.
- **Novavax** believes that MERS-CoV vaccine candidate may provide a path forward for a vaccine for this emerging threat.



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