References

1. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. Emerg Infect Dis. 2015;21:1885–6. https://doi.org/10.3201/eid2110.150847
2. US Centers for Disease Control and Prevention. 2016 case counts in the US [cited 2018 Oct 26]. https://www.cdc.gov/zika/reporting/2016-case-counts.html
3. US Centers for Disease Control and Prevention. 2017 case counts in the US [cited 2018 Oct 26]. https://www.cdc.gov/zika/reporting/2017-case-counts.html
4. Slaughter L, Keselman A, Kushniruk A, Patel VL. A framework for capturing the interactions between laypersons' understanding of disease, information gathering behaviors, and actions taken during an epidemic. J Biomed Inform. 2005;38:298–313. https://doi.org/10.1016/j.jbi.2004.12.006

Address for correspondence: Tara Kirk Sell, Johns Hopkins Bloomberg School of Public Health, 621 East Pratt St, Ste 210, Baltimore, MD 21202, USA; email: tksell@jhu.edu

Serologic Detection of Middle East Respiratory Syndrome Coronavirus Functional Antibodies

Nisreen M.A. Okba, Ivy Widjaja, Wentao Li, Corine H. GeurtsvanKessel, Elmoubasher A.B.A. Farag, Mohammed Al-Hajri, Wan Beom Park, Myoung-don Oh, Chantal B.E.M. Reusken, Marion P.G. Koopmans, Berend-Jan Bosch, Bart L. Haagmans

Author affiliations: Erasmus Medical Center, Rotterdam, the Netherlands (N.M.A. Okba, C.H. GeurtsvanKessel, C.B.E.M. Reusken, M.P.G. Koopmans, B.L. Haagmans); Utrecht University, Utrecht, the Netherlands (I. Widjaja, W. Li, B.-J. Bosch); Ministry of Public Health, Doha, Qatar (E.A.B.A. Farag, M. Al-Hajri); Seoul National University College of Medicine, Seoul, South Korea (W.B. Park, M.-d. Oh); Center for Infectious Disease Control, Bilthoven, the Netherlands (C.B.E.M. Reusken)

DOI: https://doi.org/10.3201/eid2605.190921

We developed and validated 2 species-independent protein-based assays to detect Middle East respiratory syndrome coronavirus functional antibodies that can block virus receptor-binding or sialic acid-attachment. Antibody levels measured in both assays correlated strongly with virus-neutralizing antibody titers, proving their use for serologic confirmatory diagnosis of Middle East respiratory syndrome.

The zoonotic introductions and ongoing outbreaks of Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) pose a global threat (*1*,*2*) necessitating continuous serosurveillance to monitor virus spread alongside the development of vaccine and antibodies as countermeasures. Both approaches require validated assays to evaluate specific antibody responses. Although MERS-CoV serologic assays have been developed (*2*–*6*), those detecting functional antibodies cannot be applied in all laboratories and can require Biosafety Level 3 (BSL-3) containment. Recombinant protein-based immunoassays are easier to operate and standardize and do not require BSL-3 containment. However, MERS-CoV protein-based assays developed thus far can only detect antibody binding and give no information on antibody functionality. The MERS-CoV spike protein N terminal subunit (S1) contains 2 functional domains: the N-terminal domain ($S1^A$), which binds sialic acid, the viral attachment factor; and the receptor-binding domain (RBD) ($S1^B$), which binds dipeptidyl peptidase 4, the virus receptor (*7*,*8*). Antibodies against those 2 domains can block MERS-CoV infection (*9*). Based on this fundamental knowledge, we developed 2 recombinant protein-based functional assays.

First, we developed an S1-based competitive ELISA, a receptor-binding inhibition assay (RBI), to test for antibodies that block the interaction with dipeptidyl peptidase 4, the viral receptor (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/26/5/19-0921-App1.pdf). We validated the specificity of the assay for human diagnostics using serum samples from healthy blood donors, PCR-confirmed non–coronavirus-infected patients and non–MERS-CoV–infected patients (cohorts H1–H3) (Appendix Table 1). At a 1/20 dilution, none of the samples from non–MERS-CoV-infected humans showed a ≥50% reduction in signal (RBI_{50}) (Figure, panel A), indicating a high specificity of the assay. MERS-CoV–specific RBI antibodies were detected in all the 90% plaque reduction neutralization assay ($PRNT_{90}$)–positive serum samples of the PCR-confirmed MERS-CoV patients tested (Appendix Table 2, Figure 2). The percentage reduction in signal strongly correlated with neutralizing antibody titers (Figure, panel B). The RBI_{50} assay showed similar sensitivity to the $PRNT_{90}$ assay.

Because the RBI assay is species-independent, we validated its ability to detect RBI antibodies in dromedaries. At a 1/20 dilution, none of the naive dromedary serum samples (*10*) reacted in the assay, whereas all samples from MERS-CoV–infected dromedaries (2) resulted in a >90% reduction in signal (Appendix

Table 1, Figure 3, panel A). We detected RBI antibodies in the samples of vaccinated and experimentally infected dromedaries (Appendix Figure 3, panel B). Overall, the RBI_{50} was highly specific and showed comparable sensitivity to $PRNT_{90}$ for detection of MERS-CoV–specific RBI (neutralizing) antibodies after infection and vaccination (Appendix Figure 3, panel C).

Apart from the RBD, the MERS-CoV S1 contains an α2,3 sialic acid–binding $S1^A$ domain (*7*). When this domain was multivalently presented on self-assembling lumazine synthase (LS) nanoparticles ($S1^A$-Np), it was able to hemagglutinate human erythrocytes. To generate $S1^A$-Np, we genetically fused the $S1^A$ domain to LS and expressed the particles in HEK-293S cells (Appendix Figure 4, panel

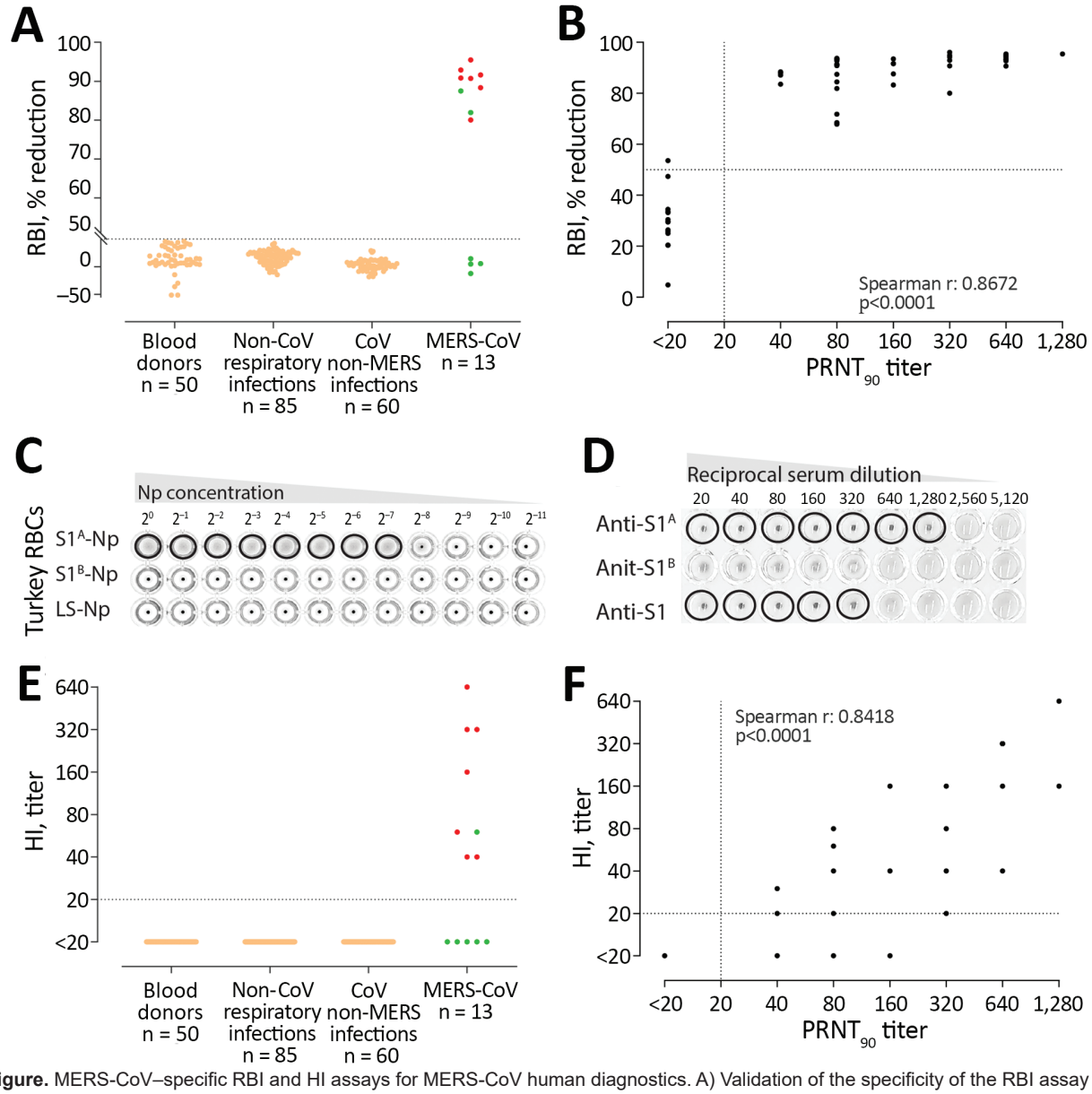


Figure. MERS-CoV–specific RBI and HI assays for MERS-CoV human diagnostics. A) Validation of the specificity of the RBI assay for the detection of MERS-CoV–specific antibodies in humans. Red dots indicate severe illness. Green dots indicate mild illness. B) Correlation between neutralizing and RBI antibody responses after MERS-CoV infection. C) Hemagglutination of turkey erythrocytes using $S1^A$-nanoparticles. $S1^A$-, $S1^B$-, or empty self-assembling lumazine synthase nanoparticles were serially diluted and tested for the ablity to agglutinate turkey RBCs. D) Specificity of the HI assay for the detection of MERS-CoV $S1^A$–directed antibodies. Rabbit anti-$S1^A$, anti $S1^B$, or anti-S1 serum samples were serially diluted and tested for the ability to block $S1^A$-nanoparticles–induced hemagglutination of turkey RBCs. E) Validation of HI assay for the detection of MERS-CoV–specific antibodies in humans. F) Scatter plot correlating $PRNT_{90}$ neutralization titers and HI titers after MERS-CoV infection. CoV, human coronavirus; HI, hemagglutination inhibition; MERS-CoV, Middle East respiratory syndrome coronavirus; $PRNT_{90}$, 90% reduction in plaque reduction neutralization test; RBI, receptor-binding inhibition.

A). By using $S1^A$-Np, we developed a hemagglutination inhibition (HI) assay to detect antibodies capable of blocking virus interaction with sialic acids (Appendix Figure 4, panel B). To set up the assay using turkey RBCs, we tested the ability of $S1^A$-Np to agglutinate turkey erythrocytes by using empty (LS)-Np and $S1^B$-Np as negative controls. Although neither the lumazine synthase–Np nor the $S1^B$-Np showed any hemagglutination at any temperature tested, the $S1^A$-Np induced hemagglutination at 4°C; we also noted hemagglutination when using dromedary erythrocytes (Figure, panel C; Appendix Figure 4, panel C). Although antibodies against the S1 and $S1^A$ domain inhibited hemagglutination showing high HI titers, $S1^B$ antibodies were negative for HI (Figure, panel D).

Next, we used the same cohort of serum samples for validating the RBI assay. None of the samples from healthy blood donors, PCR-confirmed non–coronavirus-infected and non–MERS-CoV–infected patients (cohorts H1–H3) showed any HI at the 1/20 dilution (Figure, panel E). HI antibodies were detected in the samples of all severely infected MERS-CoV patients and that of 1 mildly infected MERS-CoV patient (Figure, panel E; Appendix Figure 5); only 2 of the mildly infected MERS-CoV patients were $PRNT_{90}$-positive (Appendix Table 2). Serum HI titers correlated strongly with neutralizing antibody titers detected by a whole virus neutralization assay ($PRNT_{90}$); nonetheless, the $PRNT_{90}$ assay was more sensitive (Figure, panel F). Similarly, only serum samples from MERS-CoV–infected dromedaries were HI-positive (10/13), whereas none of the naive dromedary camel serum samples showed any HI (Appendix Figure 6, panel A). HI antibodies were detected in serum samples of vaccinated dromedaries after booster immunization (Appendix Figure 6, panel B). Overall, although less sensitive, the antibody titers detected by the HI assay correlated strongly with the neutralizing antibody titers detected by $PRNT_{90}$ assay (Appendix Figure 6, panel C).

The RBI and HI assays we developed are easy to operate and standardize and can detect functional antibodies against 2 MERS-CoV S1 domains responsible for virus entry (RBD) and attachment ($S1^A$). Both assays are protein-based and can be carried out in a 96-well plate format, therefore providing BSL-1 high-throughput platforms. The assays can be used as confirmatory assays for human and dromedary MERS-CoV diagnostics and for antibody and vaccine evaluation.

This work was supported by the Zoonoses Anticipation- and Preparedness Initiative (ZAPI project; Innovative Medicines Initiative grant agreement no. 115760), with the assistance and financial support of Innovative Medicines Initiative and the European Commission, in-kind contributions from European Federation of Pharmaceutical Industries and Associations partners. Proteins used for the assays described in this study can be provided by European Virus Archive–global. Requests should be submitted on the European Virus Archive–global website (http://www.european-virus-archive.com).

About the Author

Ms. Okba is a PhD candidate in the Viroscience Department at Erasmus Medical Center. Her research interests include the development of diagnostic and intervention strategies for emerging viruses.

References

1. World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) [cited 2019 May 10]. http://www.who.int/emergencies/mers-cov
2. Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis. 2014;14:140–5. https://doi.org/10.1016/S1473-3099(13)70690-X
3. Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill. 2012;17:20334. https://doi.org/10.2807/ese.17.49.20334-en
4. Reusken C, Mou H, Godeke GJ, van der Hoek L, Meyer B, Müller MA, et al. Specific serology for emerging human coronaviruses by protein microarray. Euro Surveill. 2013;18:20441. https://doi.org/10.2807/1560-7917.ES2013.18.14.20441
5. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, et al. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. Euro Surveill. 2013;18:20574. https://doi.org/10.2807/1560-7917.ES2013.18.36.20574
6. Trivedi S, Miao C, Al-Abdallat MM, Haddadin A, Alqasrawi S, Iblan I, et al. Inclusion of MERS-spike protein ELISA in algorithm to determine serologic evidence of MERS-CoV infection. J Med Virol. 2018;90:367–71. https://doi.org/10.1002/jmv.24948
7. Li W, Hulswit RJG, Widjaja I, Raj VS, McBride R, Peng W, et al. Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein. Proc Natl Acad Sci U S A. 2017;114:E8508–17. https://doi.org/10.1073/pnas.1712592114
8. Mou H, Raj VS, van Kuppeveld FJ, Rottier PJ, Haagmans BL, Bosch BJ. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J Virol. 2013;87:9379–83. https://doi.org/10.1128/JVI.01277-13

9. Widjaja I, Wang C, van Haperen R, Gutiérrez-Álvarez J, van Dieren B, Okba NMA, et al. Towards a solution to MERS: protective human monoclonal antibodies targeting different domains and functions of the MERS-coronavirus spike glycoprotein. Emerg Microbes Infect. 2019;8:516–30. https://doi.org/10.1080/22221751.2019.1597644
10. Haagmans BL, van den Brand JM, Raj VS, Volz A, Wohlsein P, Smits SL, et al. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science. 2016;351:77–81. https://doi.org/10.1126/science.aad1283

Address for correspondence: Berend-Jan Bosch, Virology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL, Utrecht, the Netherlands; email: b.j.bosch@uu.nl; Bart L. Haagmans, Department of Viroscience, Erasmus Medical Center, PO Box 2040, 3000 CA, Rotterdam, the Netherlands; email: b.haagmans@erasmusmc.nl

Novel *Ehrlichia* Strain Infecting Cattle Tick *Amblyomma neumanni*, Argentina, 2018

Lucía Fargnoli, Camilo Fernandez, Lucas D. Monje

Author affiliation: Instituto de Ciencias Veterinarias del Litoral, UNL-CONICET, Esperanza, Argentina

DOI: https://doi.org/10.3201/eid2605.190940

In 2018, we detected a novel *Ehrlichia* strain infecting *Amblyomma neumanni* ticks in Argentina. The novel strain is phylogenetically related to the ruminant pathogen *E. ruminantium* and represents a potential risk for veterinary and public health because *A. neumanni* ticks parasitize domestic and wild ruminants and bite humans.

etymologia

Coronavirus [kə-ro'nə-vi"rus]

Ronnie Henry

The first coronavirus, avian infectious bronchitis virus, was discovered in 1937 by Fred Beaudette and Charles Hudson. In 1967, June Almeida and David Tyrrell performed electron microscopy on specimens from cultures of viruses known to cause colds in humans and identified particles that resembled avian infectious bronchitis virus. Almeida coined the term "coronavirus," from the Latin *corona* ("crown"), because the glycoprotein spikes of these viruses created an image similar to a solar corona.

Strains that infect humans generally cause mild symptoms. However, more recently, animal coronaviruses have caused outbreaks of severe respiratory disease in humans, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease (COVID-19).

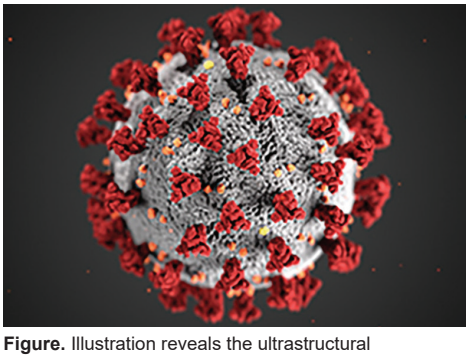


Figure. Illustration reveals the ultrastructural morphology exhibited by coronaviruses. Note the spikes that adorn the outer surface of the virus, which impart the look of a corona surrounding the virion, when viewed electron microscopically. Photo: CDC/ Alissa Eckert, MS; Dan Higgins, MAMS

Sources

1. Almeida JD, Tyrrell DA. The morphology of three previously uncharacterized human respiratory viruses that grow in organ culture. J Gen Virol. 1967;1:175–8. https://doi.org/10.1099/0022-1317-1-2-175
2. Beaudette FR, Hudson CB. Cultivation of the virus of infectious bronchitis. J Am Vet Med Assoc. 1937;90:51–8.
3. Estola T. Coronaviruses, a new group of animal RNA viruses. Avian Dis. 1970;14:330–6. https://doi.org/10.2307/1588476
4. Groupe V. Demonstration of an interference phenomenon associated with infectious bronchitis virus of chickens. J Bacteriol. 1949;58:23–32. https://doi.org/10.1128/JB.58.1.23-32.1949

Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop V18-2, Atlanta, GA 30329-4027, USA; email: boq3@cdc.gov

DOI: https://doi.org/10.3201/eid2605.ET2605

Article DOI: https://doi.org/10.3201/eid2605.190921

Serologic Detection of Middle East Respiratory Syndrome Coronavirus Functional Antibodies

Appendix

Materials and Methods

Serum Samples

To validate the specificity of the developed assays for the detection of MERS-CoV-specific antibodies in humans, we used a set of sera we previously described in an earlier study to validate a MERS-CoV specific S1 ELISA (*1*) (Appendix Table 1, cohorts H1–H5). We also included sera from MERS-CoV infected camels (*2*) as well as sera from MERS-CoV vaccinated camels (*3*) to validate the use of the assays for camel MERS-CoV diagnostics and evaluation of immune responses following vaccination (Appendix Table 1, sample sets D1–D3). The use of human sera from the Netherlands was approved by the local medical ethical committee (MEC approval no. 2014–414) and from South Korea by the Institutional Ethics Review Board of Seoul National University Hospital (approval no. 1506–093–681). The use of camel sera was approved as previously described (*2*,*3*)

Protein Production

For the receptor binding inhibition assay, both DPP4 and MERS-S1-mFc were produced in HEK-293T cells as previously described. The DPP4 ectodomain (39–766) was expressed as an N terminally strep-tagged protein and purified from cell culture supernatant using Strep-Tactin sepharose beads (IBA GmbH). MERS-CoV S1 (1–751) was C-terminally fused to a mouse IgG2a Fc in pCAGGS expression vector and purified from cell culture supernatant using protein A sepharose beads.

For the hemagglutination inhibition assay, empty lumazine synthase nanoparticles (LS-Np) were produced as previously described (*4*). $S1^A$ nanoparticles ($S1^A$-Np) were produced by genetically linking the $S1^A$ encoding region of the MERS-CoV spike (amino acids19–357; EMC

strain GenBank Acc. no. JX869059.2) to the lumazine synthase encoding gene in pCAGGS vector encoding a CD5 signal peptide and strep tag. $S1^A$-Np were expressed in HEK-293S cells and purified using Strep-Tactin sepharose beads.

Receptor Binding Inhibition Assay

We developed a competitive ELISA to detect antibodies capable of blocking of the binding of MERS-CoV to its cellular receptor DPP4. ELISA plates were coated overnight at 4°C with 2 ug/ml recombinant DPP4 in PBS. The plates were washed with PBS and blocked with 3% BSA/PBS-0.5% tween-20 for 1 hr at room temp. In the meantime, 1/20 diluted sera (or further 2-fold serially diluted for titer determination) were mixed with 5 ng of S1-mFc in a total volume of 100 ul blocking buffer per well and incubated for 1 hr at room temperature. Wells with no serum (only S1-mFc) were included in each run to calculate to maximum binding. Following 1 hr of incubation the mix was transferred to blocked plates and allowed to incubate for 1 hr further. The plates were washed 3 times with PBS/0.05% tween-20, and the amount of S1-mFc bound to the plate was determined by adding HRP-labeled anti-mouse IgG (1:2000, Dako) and incubating for 1 hr. Following washing, the signal was revealed by adding 100 ul of TMB and the reaction was stopped using sulfuric acid. Absorbance was measured using Tecan. Blocking was determined as percentage reduction of the sample signal from the blank signal (no serum). A 50% reduction in signal (RBI_{50}) in a ≥1/20 diluted sample was considered positive. Serum antibody titers were determined as the reciprocal of the highest serum dilution resulting in a ≥50% signal reduction.

Hemagglutination Assay

We tested the ability of $S1^A$ multivalently-expressed on lumazine synthase nanoparticles ($S1^A$-Np) to agglutinate turkey and dromedary RBCs.

For the HA assay, fifty µl of 2-fold serially diluted Nps were mixed with an equal volume of 0.5% RBCs in PBS and incubated for 1 hr at 4°C. Following incubation, the hemagglutination activity was assessed and the HA titer (HA units; HAU) of the Nps was recorded as the dilution of the last well showing hemagglutination.

Hemagglutination Inhibition Assay

To carry out the hemagglutination inhibition assay, sera were 2-fold serially diluted starting at a 1/10 dilution in a total volume of 50 ul PBS. $S1^A$-Nps corresponding to 4 HAU in 25 ul PBS were added to each well and the mix was incubated for 30 min at 37°C. Following

incubation, 25 ul of 0.5% turkey RBCs in PBS were added and further incubated for 1 hr at 4°C after which the serum HI titer was scored. A serum titer ≥20 was considered positive.

Plaque Reduction Neutralization Assay (PRNT)

All sera included in this study were previously tested for MERS-CoV neutralization using the $PRNT_{90}$ assay (*1*–*3*). Owing to the specificity and sensitivity, neutralization assays are considered the gold standard for MERS-CoV serology. Thus, we compared the performance of the developed assays RBI and HI to $PRNT_{90}$ to assess their specificity and sensitivity.

References

1. Okba NMA, Raj VS, Widjaja I, GeurtsvanKessel CH, de Bruin E, Chandler FD, et al. Sensitive and specific detection of low-level antibody responses in Mild Middle East respiratory syndrome coronavirus infections. Emerg Infect Dis. 2019;25:1868–77. PubMed https://doi.org/10.3201/eid2510.190051

2. Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis. 2014;14:140–5. PubMed https://doi.org/10.1016/S1473-3099(13)70690-X

3. Haagmans BL, van den Brand JM, Raj VS, Volz A, Wohlsein P, Smits SL, et al. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science. 2016;351:77–81. PubMed https://doi.org/10.1126/science.aad1283

4. Li W, Hulswit RJG, Widjaja I, Raj VS, McBride R, Peng W, et al. Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein. Proc Natl Acad Sci U S A. 2017;114:E8508–17. PubMed https://doi.org/10.1073/pnas.1712592114

Appendix Table 1. Sample sets used in this study*

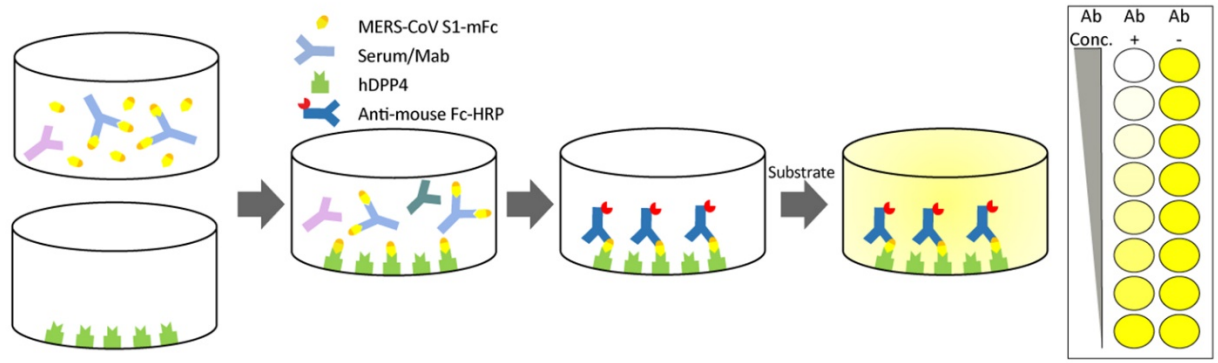
Species	Country	Cohort	Sample source		No. samples	Range (post diagnosis)	Reference
Human	The Netherlands	H1	Blood Donors (Negative cohort)		50	NA	(*1*)
		H2	Non-hCoV Respiratory Infections (N = 85)	Adenovirus	5	2- 4 w	
				Bocavirus	2		
				enterovirus	2		
				HMPV	9		
				Influenza A	13		
				Influenza B	6		
				Rhinovirus	9		
				RSV	9		
				PIV-1	4		
				PIV-3	4		
				M. pneumoniae	1		
				CMV	9		
				EBV	12		
		H3	Recent hCoV infections (N = 60)	α-CoV HCoV-229E	19	>2w –1y	
				HCoV-NL63	18		
				β-CoV HCoV-OC43	23		
		H4†	RT-PCR confirmed MERS cases	Acute	21	1–14 d	
				Convalescent	7	15–228 d	
	South Korea	H5	(n = 60 longitudinal specimen from 13 patients)	Mild infection‡	17	6–12 mo	
				Severe infection§	15	6–12 mo	
Dromedary camels	Qatar	D1	MERS-CoV seropositive		13	NA	(*2*)
	Canary Islands	D2	MERS-CoV seronegative (longitudinal specimen)	Vaccinated¶	28	0–63 dpv	(*3*)
		D3	MERS-CoV infected (longitudinal specimen)	Infected#	28	0–14 dpi	

*CoV, coronavirus; CMV, Cytomegalovirus; d, day; dpi, days post-infection; dpv, days post-vaccination; EBV, Epstein-Barr virus; HCoV, human coronavirus; HMPV, Human metapneumovirus; MERS, Middle East respiratory syndrome; mo, month; NA, not applicable; PIV, parainfluenza virus; RSV, respiratory syncytial virus; w, week; y, year.
†Samples taken from 2 case-patients at different time points.
‡Samples taken from 6 case-patients at different time points.
§Samples taken from 5 case-patients at different time points.
¶Samples taken from 4 camels at different time points.
#Samples taken from 4 camels at different time points.

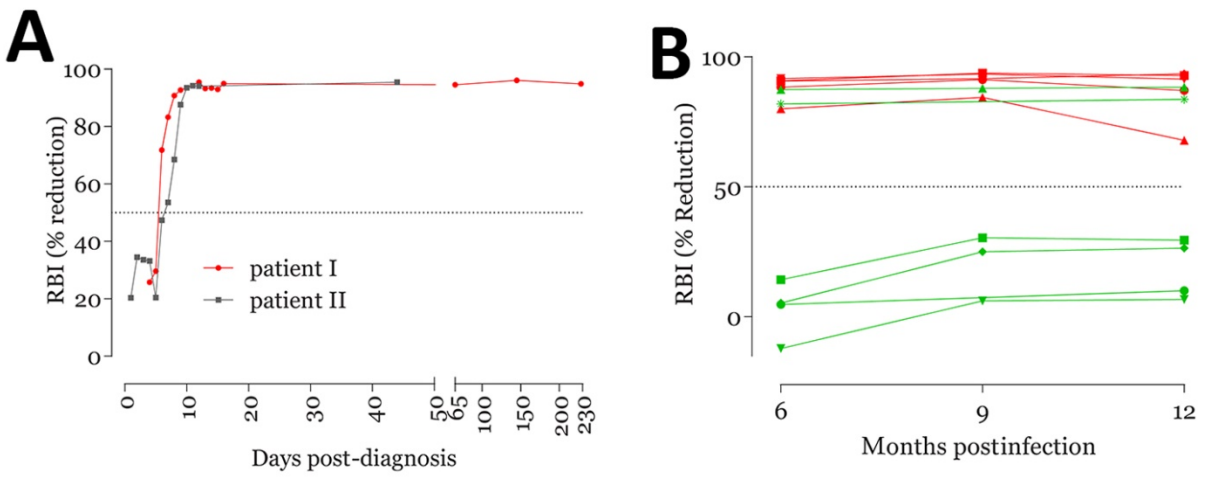
Appendix Table 2. Comparative validation results of the RBI and HI assays versus the $PRNT_{90}$*

Species	Cohort	Sample source		No. samples	N positives / N tested				Validation aspect
					RBI_{50}		HI		
					$PRNT_{90}$ positive	$PRNT_{90}$ negative	$PRNT_{90}$ positive	$PRNT_{90}$ negative	
Human	H1	Blood Donors		50	NA	0/50	NA	0/50	Specificity
	H2	Non-CoV Respiratory Infections (N = 85)	Adenovirus	5	NA	0/5	NA	0/5	
			Bocavirus	2	NA	0/2	NA	0/2	
			Enterovirus	2	NA	0/2	NA	0/2	
			HMPV	9	NA	0/9	NA	0/9	
			Influenza A	13	NA	0/13	NA	0/13	
			Influenza B	6	NA	0/6	NA	0/6	
			Rhinovirus	9	NA	0/9	NA	0/9	
			RSV	9	NA	0/9	NA	0/9	
			PIV-1	4	NA	0/4	NA	0/4	
			PIV-3	4	NA	0/4	NA	0/4	
			M. pneumoniae	1	NA	0/1	NA	0/1	
			CMV	9	NA	0/9	NA	0/9	
			EBV	12	NA	0/12	NA	0/12	
	H3	Recent CoV infections (N = 60)	α-CoV HCoV-229E	19	NA	0/19	NA	0/19	
			HCoV-NL63	18	NA	0/18	NA	0/18	
			β-CoV HCoV-OC43	23	0/2	0/23	0/2	0/23	
	H4†	RT-PCR confirmed	≤14 d post diagnosis	21	11/11	1/10	7/11	0/10	Sensitivity
			>14 d post diagnosis	7	7/7	NA	7/7	NA	
	H5	MERS cases (n = 60 longitudinal specimen from 13 patients)	Mild infection‡	17	5/5	0/12	2/5	0/12	
			Severe infection§	15	15/15	NA	15/15	NA	
Dromedary camels	D1	MERS-CoV seropositive		13	13/13	NA	10/13	NA	
	D2	MERS-CoV seronegative (longitudinal specimen)	Vaccinated¶	28	16/20	0/8	16/20	0/8	
	D3		Infected#	28	5/6	0/22	0/6	0/22	

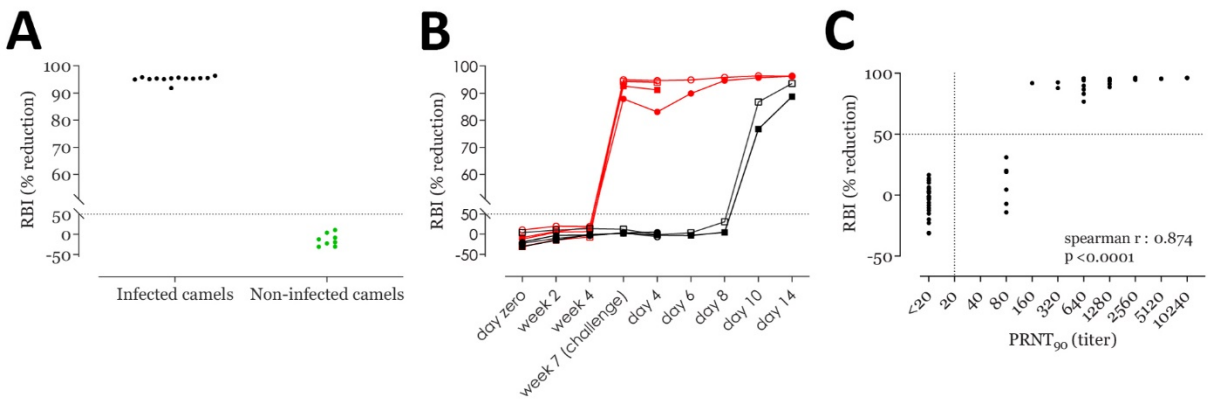
*CoV, coronavirus; CMV, Cytomegalovirus; EBV, Epstein-Barr virus; HCoV, human coronavirus; HMPV, Human metapneumovirus; MERS, Middle East respiratory syndrome; NA, not applicable; PIV, parainfluenza virus; RSV, respiratory syncytial virus.
†Samples taken from 2 case-patients at different time points.
‡Samples taken from 6 case-patients at different time points.
§Samples taken from 5 case-patients at different time points.
¶Samples taken from 4 camels at different time points.
#Samples taken from 4 camels at different time points.



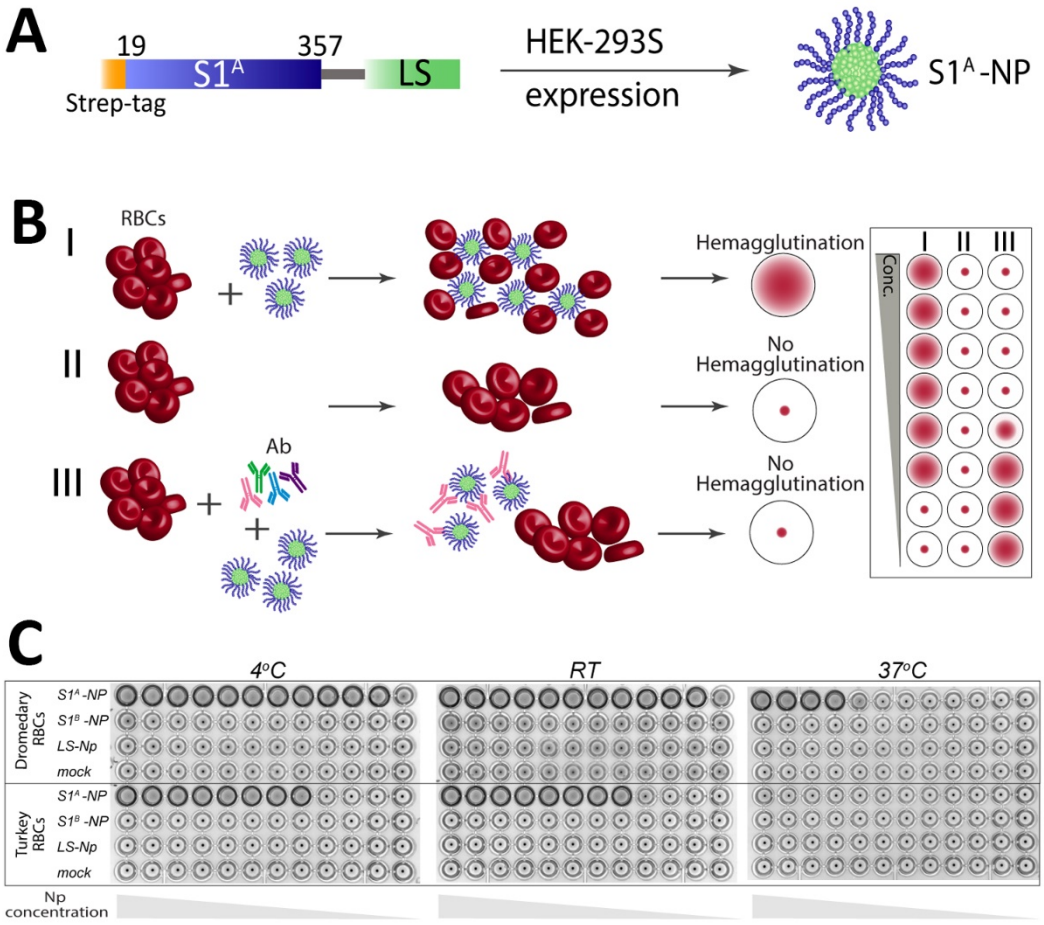
Appendix Figure 1. Schematic diagram showing the principle of the receptor binding inhibition (RBI) assay. S1-mFc, MERS-CoV S1 protein with a mouse Fc tag; hDPP4, human Dipeptidyl Peptidase-4 (MERS-CoV receptor); HRP, horse radish peroxidase.



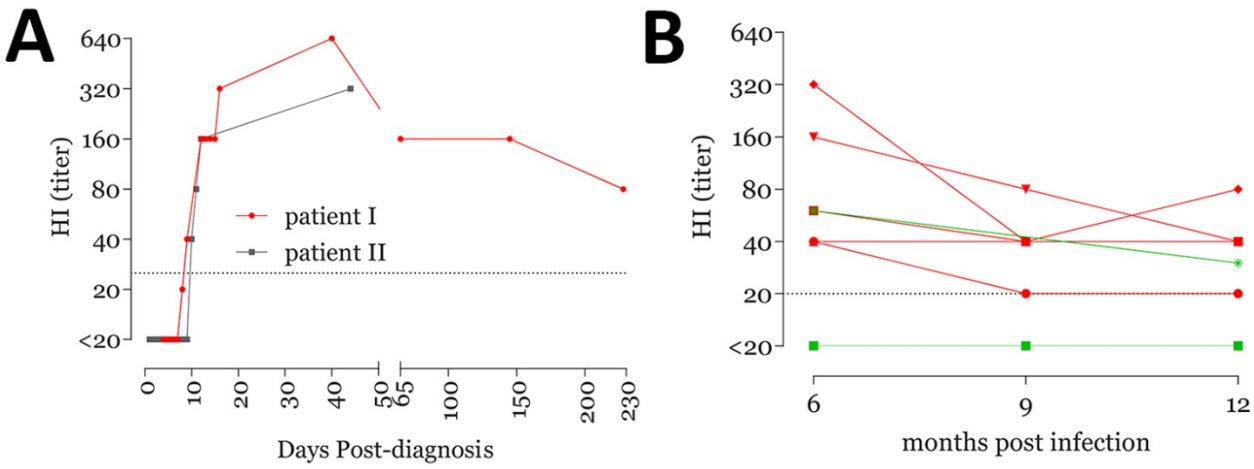
Appendix Figure 2. Kinetics of RBI antibody responses in PCR-confirmed MERS patients. RBI antibody responses in (A) two acute to convalescent phase patients and in (B) severe (red, n = 5) and mild (green, n = 6) MERS-CoV patients six to twelve months post-infection.



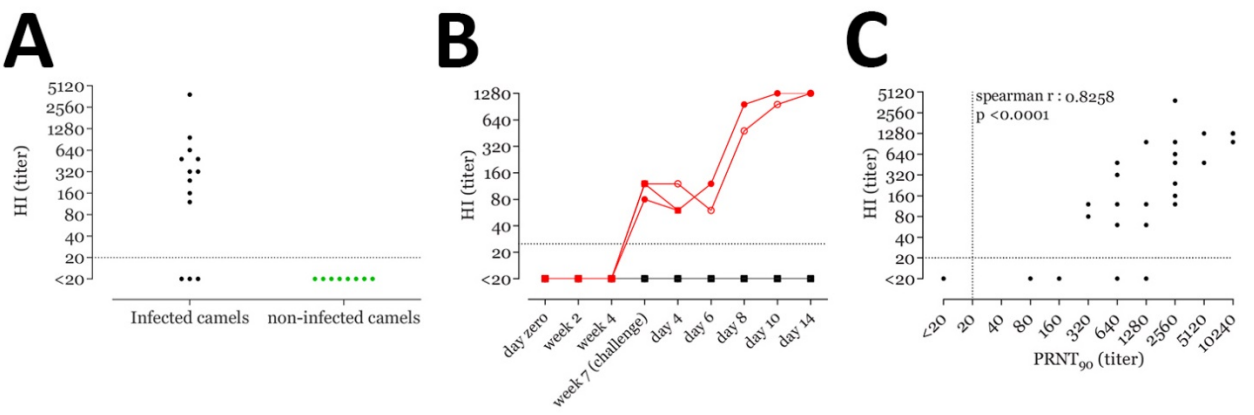
Appendix Figure 3. MERS-CoV specific receptor binding inhibition (RBI) assay for MERS-CoV dromedary camel diagnostics. A)Validation of the specificity of the RBI assay for the detection of MERS-CoV specific antibodies in the sera of MERS-CoV-infected (black) and naïve (green) dromedary camels. B) Kinetics of RBI antibody responses in dromedary camels following vaccination (red) and infection (black). C) Correlation between neutralizing and RBI antibody responses following MERS-CoV infection or vaccination in dromedaries. $PRNT_{90}$, 90% reduction in plaque reduction neutralization test.



Appendix Figure 4. Development of MERS-CoV HI assay. A) Schematic diagram of the production of $S1^A$ lumazine synthase (LS) nanoparticles (Np). B) The principle of the HI assay showing the hemagglutination of red blood cells (RBCs) in the presence of $S1^A$-Np(I), no-hemagglutination in the absence of the particles (II) and the inhibition of hemagglutination (HI) by $S1^A$-directed antibodies (III). C) $S1^A$-Np induced hemagglutination of dromedary and turkey RBCs at different temperatures.



Appendix Figure 5. Kinetics of HI antibody responses in PCR-confirmed MERS patients. HI antibody titers in (A) two acute to convalescent phase patients and in (B) six severe (red) and five mild (green) MERS-CoV patients six to twelve months post-infection.



Appendix Figure 6. MERS-CoV hemagglutination inhibition (HI) assay for MERS-CoV dromedary camel diagnostics. A) Validation of the specificity of the HI assay for the detection of MERS-CoV specific antibodies in the sera of MERS-CoV-infected (black) and naïve (green) dromedary camels. B) Kinetics of HI antibody responses in dromedary camels following vaccination (red) and infection (black). C) Correlation between neutralizing and HI antibody responses following MERS-CoV infection or vaccination in dromedaries. $PRNT_{90}$, 90% reduction in plaque reduction neutralization test.