



**HAL**  
open science

## Heat inactivation of the Middle East respiratory syndrome coronavirus

India Leclercq, Christophe Batéjat, Ana Burguière, Jean-Claude Manuguerra

► **To cite this version:**

India Leclercq, Christophe Batéjat, Ana Burguière, Jean-Claude Manuguerra. Heat inactivation of the Middle East respiratory syndrome coronavirus. *Influenza and Other Respiratory Viruses*, 2014, 8 (5), pp.585-586. 10.1111/irv.12261 . pasteur-03915081

**HAL Id: pasteur-03915081**

**<https://pasteur.hal.science/pasteur-03915081>**

Submitted on 29 Dec 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

# Heat inactivation of the Middle East respiratory syndrome coronavirus

India Leclercq,<sup>a,b</sup> Christophe Batéjat,<sup>a</sup> Ana M. Burguière,<sup>a</sup> Jean-Claude Manuguerra<sup>a</sup>

<sup>a</sup>Laboratory for Urgent Response to Biological Threats (CIBU), Environment and Infectious Risks Unit, Institut Pasteur, Paris, France. <sup>b</sup>Université Paris Diderot, Sorbonne Paris Cité (Cellule Pasteur), Paris, France.

Correspondence: India Leclercq, Laboratory for Urgent Response to Biological Threats (CIBU), Environment and Infectious Risks Unit, Institut Pasteur, 28 rue du docteur Roux, 75724 Paris, Cedex 15, France. E-mail: india.leclercq@pasteur.fr

Accepted 8 May 2014. Published Online 24 June 2014.

The culture supernatants of the emerging Middle East respiratory syndrome coronavirus (MERS-CoV) were submitted to three temperatures over time and tested for infectivity by TCID<sub>50</sub> method on Vero E6 cells. At 56°C, almost 25 minutes were necessary to reduce the initial titre by 4 log<sub>10</sub>. Increasing temperature to 65°C had a strong negative effect on viral infectivity as virucidity dropped

significantly to 1 minute. On the contrary, no significant decrease in titre was observed after 2 hours at 25°C. These data might be useful in establishing biosafety measures in laboratories against MERS-CoV.

**Keywords** Heat, inactivation, MERS-CoV.

Please cite this paper as: India Leclercq *et al.* (2014) Heat inactivation of the Middle East respiratory syndrome coronavirus. *Influenza and Other Respiratory Viruses* 8(5), 585–586.

## Short report

The emerging Middle East respiratory syndrome coronavirus (MERS-CoV) was found to cause sporadic cases of severe acute respiratory infection. Between April 2012 and 26 April 2014, a total of 261 laboratory-confirmed cases of infection with MERS-CoV were reported, including 93 deaths, in nine different countries.<sup>1</sup> To date, the viral transmission route is still not elucidated, although recent studies showed that tomb bats and camels may play a role as reservoirs or intermediate hosts.<sup>2,3</sup> Coronaviruses are enveloped viruses, usually known to be fragile in the environment. However, enveloped viruses can persist in the environment for extended periods of time, even at 35°C.<sup>4</sup> Understanding

the potential effect of heat inactivation of the novel coronavirus is of significant value to elaborate proper public intervention measures.

A human strain of MERS-CoV was isolated in our laboratory from a French patient hospitalised in June 2013 after a nosocomial transmission.<sup>5</sup> The viral strain MERS-CoV Hu/France–FRA2\_130569/2013 (FRA2) was grown on MRC5 cells (RD-Biotech REF-84002) for the first passage and on Vero E6 cells (ATCC<sup>®</sup> CRL-1586) for the second passage. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM 1X, GIBCO; Invitrogen, Saint Aubin, France) and supplemented with 5% foetal calf serum (FCS), antibiotics (0.1 units penicillin, 0.1 mg streptomycin per ml, GIBCO; Invitrogen) at 37°C in humidified 5% CO<sub>2</sub> incubator.

**Table 1.** TCID<sub>50</sub> per ml values obtained at 56 and 65°C

Time (minute)	56°C					65°C				
	0.5	15	30	60	120	0.5	15	30	60	120
Sample 1	10 <sup>5.5</sup>	10 <sup>0.67</sup>	ND	ND	ND	10 <sup>4.67</sup>	ND	ND	ND	ND
Sample 2	10 <sup>6.17</sup>	10 <sup>1.0</sup>	10 <sup>0.67</sup>	ND	ND	10 <sup>2.00</sup>	ND	ND	ND	ND
Sample 3	10 <sup>4.67</sup>	ND	10 <sup>1.33</sup>	ND	10 <sup>0.67</sup>	10 <sup>3.67</sup>	ND	ND	ND	ND

Time zero values were 10<sup>5.59</sup> TCID<sub>50</sub> per ml for each sample. ND: not detected (below the limit of virus detection which corresponded to 10<sup>0.67</sup> TCID<sub>50</sub> per ml). The whole experiment was performed twice with similar results (data not shown).

**Table 2.** Persistence times estimated using linear regression model for heat inactivation at 25, 56 and 65°C

Strain	Temperature	Slope (a)	y-Intercept (b)	x-Intercept (minute)	Virucidity (minute)
FRA2	25°C	0.006	5.25	NA	NA
	56°C	-0.16	5.09	30.7 [26.0; 35.2]	24.1
	65°C	-3.62	5.25	1.45 [1.0; 1.9]	1.1

Slope (a), y-intercept (b) and x-intercept values of the linear regression straight lines calculated from TCID<sub>50</sub> values (ND values in Table 1 were not included in the calculation). Virucidity corresponded to the duration necessary to obtain a fourfold reduction of the titre in log<sub>10</sub>. The values in brackets corresponded to the 99% confidence intervals. NA, not applicable. Each condition was performed in triplicates. The whole experiment was performed twice with similar results (data not shown).

The cell culture supernatant was used for inactivation assays and whole-genome sequencing (manuscript under submission). Culture supernatants (500 µl) with a titre of 10<sup>5-59</sup> TCID<sub>50</sub> per ml were submitted to three temperatures over time and tested for infectivity by TCID<sub>50</sub> method on Vero E6 cells as described previously, except that examination for cytopathic effect was performed after 6 days.<sup>4</sup> Several time points were chosen (0, 0.5, 15, 30, 60 and 120 minutes). Each condition was performed in triplicates, and the whole experiment was accomplished twice. Experimental data from one experiment are shown in Table 1. For each condition, we determined the virucidal activity of heat at 56 and 65°C, which corresponded to a reduction of 4 log<sub>10</sub> of the titre according to the European Standards (NF EN 14476 available at <http://www.afnor.org/>; Table 2). At 56°C, which is the common temperature used for inactivation of enveloped viruses, such as influenza viruses, and serum decomplexation, almost 25 minutes were necessary to reduce the initial titre by 4 log<sub>10</sub>. Increasing temperature to 65°C had a strong negative effect on viral infectivity as virucidity dropped significantly to 1 minute. Fifteen minutes at 65°C is more than sufficient to totally inactivate the sample. By contrast, no decrease in titre was observed after 2 hours at 25°C.

Serum heat inactivation at 56°C for 30 minutes is a standard procedure in diagnostic laboratories to eliminate the potential complement interference in serological assays. Our results showed that this procedure is sufficient for viral inactivation as virus titres in blood are expected to be weaker than at the point of infection. This would be also sufficient for inactivation of viruses present in lower respiratory specimens, which are now recommended by WHO rather than nasopharyngeal swabs for viral diagnosis. For example, the FRA2 original clinical specimen (induced sputum) contained 6.5 × 10<sup>7</sup> genome

copies per ml for Orf1a calculated from the C<sub>t</sub> values using a standard (manuscript under submission). These data might be also useful in establishing biosafety measures in laboratories against MERS-CoV.

## Acknowledgements

The research leading to these results has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under Grant Agreement N° 278433-PREDEMICS. We are also grateful to BNP-Paribas and its foundation (<http://www.mecenat.bnpparibas.com/>) for their financial support. We also thank Gilberte Coralie, Frederic Fichenick and Claudine Rousseaux for helping.

## References

- 1 WHO. Middle East respiratory syndrome coronavirus (MERS-CoV) – update [Internet]. WHO. Available at [http://www.who.int/csr/don/2014\\_04\\_26\\_mers/en/](http://www.who.int/csr/don/2014_04_26_mers/en/) (Accessed 28 April 2014).
- 2 Memish ZA, Mishra N, Olival KJ *et al.* Middle East respiratory syndrome coronavirus in Bats, Saudi Arabia. *Emerg Infect Dis* 2013; 19:1819–1823.
- 3 Reusken CB, Haagmans BL, Müller MA *et al.* Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *Lancet Infect Dis* 2013; 13:859–866.
- 4 Dublineau A, Batéjat C, Pinon A, Burguière AM, Leclercq I, Manuguerra J-C. Persistence of the 2009 pandemic influenza A (H1N1) virus in water and on non-porous surface. *PLoS ONE* 2011; 6:e28043.
- 5 Guery B, Poissy J, el Mansouf L, *et al.* Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. *Lancet* 2013; 381:2265–2272.