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Published in *Journal of General Virology*, November 2016 97: 2799-2808 by  
Microbiology Society. The original publication is available at:  
[10.1099/jgv.0.000610](https://doi.org/10.1099/jgv.0.000610)**

# Biosafety standards for working with Crimean-Congo haemorrhagic fever virus

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**Running Title:** Crimean-Congo haemorrhagic fever virus biosafety

**Keywords:** Crimean-Congo haemorrhagic fever virus biosafety

Word count abstract: 132 words

Word count text: 3325 words

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**Abstract**

In countries from which Crimean-Congo haemorrhagic fever (CCHF) is absent, the causative virus CCHF virus (CCHFV) is classified as a hazard group 4 agent and handled in containment level 4. In contrast, most endemic countries out of necessity have had to perform diagnostic tests under biosafety level (BSL) 2 or 3 conditions. In particular, Turkey and several of the Balkan countries have safely processed more than 100000 samples over many years in BSL-2 laboratories. It is therefore advocated that biosafety requirements for CCHF diagnostic procedures should be revised, to allow the required tests to be performed under enhanced BSL-2 conditions with appropriate biosafety laboratory equipment and personal protective equipment used according to standardized protocols in the affected countries. Downgrading of CCHFV research work from Cl-4,BSL-4 to Cl-3 ,BSL-3 should also be considered.

15        **Introduction**

16        Crimean-Congo haemorrhagic fever virus (CCHFV), a member of the *Nairovirus* genus of the  
17        family *Bunyaviridae*, causes a tick-borne zoonotic infection (Crimean-Congo haemorrhagic  
18        fever (CCHF)) in parts of Africa and Eurasia [1]. CCHFV has been classified as a hazard group 4  
19        pathogen (UK) or risk group 4 (Europe, USA, international) in countries that have promulgated  
20        biosafety regulations, and should accordingly be handled in containment level 4 (CL4, UK) or  
21        biosafety level 4 (BSL-4, Europe, USA, international) laboratories (Table 1).

22        Signs and symptoms after a sudden onset of disease 1–7 days post infection, progress from  
23        high grade of fever, headache, fatigue, myalgia, abdominal pain, nausea, vomiting, diarrhoea,  
24        thrombocytopenia and rash, to haemorrhages from various body sites, shock and death in  
25        severe cases. Reported mortality rates vary widely from to 2-30% across studies and endemic  
26        countries [2,3].

27        Apart from transmission by tick bite as a major route of infection, transmission can also occur  
28        through handling or squashing of infected ticks, and contact with the blood of viraemic  
29        animals, or blood and other body fluids of patients. Consequently, livestock farmers, abattoir  
30        and healthcare workers (HCWs) dominate the literature on reported infections. Nosocomial  
31        transmission to HCWs in close contact with patients in the acute phase have been  
32        documented throughout the endemic areas and are often linked to breaches of, or non-  
33        existent, barrier nursing techniques, or to percutaneous needlestick injuries [4].

34        Following the occurrence of the first recognized outbreaks of ‘Crimean haemorrhagic fever’ in  
35        soldiers and displaced persons exposed to ticks while sleeping outdoors in 1944 and 1945,  
36        there were similar outbreaks associated with exposure of large numbers of people to ticks in  
37        major land reclamation or resettlement schemes in parts of the former Soviet Union,  
38        culminating in an epidemic in Khazakstan in 1989 [5] [6] [7]. Subsequently, there were reports

39 of a series of lesser outbreaks associated with exposure of people to blood and ticks from  
40 slaughter animals imported from Africa and Asia into the Near East [8]. Further large-scale  
41 outbreaks that occurred during the late 1990s and early 2000s, involved exposure of war  
42 refugees to outdoor conditions in Kosovo, Albania, Macedonia and the Afghanistan-Pakistan  
43 border area [7,9]. Finally, an outbreak of unprecedented magnitude emerged in Turkey in  
44 2003 with 9787 clinical and laboratory confirmed CCHF cases by 2015. This outbreak has been  
45 ascribed to an increase in the tick population triggered by climate change, altered grazing  
46 practices and prohibition of the hunting of wild hosts of ticks (Vatansever et al., 2007).

47 Consequently, in recent years the existing laboratory and health care facility infrastructure in  
48 south-eastern Europe and the Balkans, and especially in Turkey, had to adapt to deal with a  
49 large influx of patients and samples potentially infected with a hazard group 4 pathogen.

50 The purpose of this paper is to review experiences of HCWs and scientists in handling CCHF  
51 patients and CCHFV-positive materials in order to derive safe recommendations for safe  
52 laboratory processing of known or suspected CCHFV-infected samples, and particularly at  
53 which biosafety level CCHFV material and samples from CCHF patients can be handled safely.

54 First of all we re-appraise CCHF case fatality rates in endemic countries and in clinical cases.  
55 This is followed by a review of nosocomial infections and the most recent data from the large  
56 epidemic in Turkey, which indicate CCHFV is less easily transmitted from person to person than  
57 thought as exemplified by seroprevalence studies amongst health care workers dealing with  
58 CCHF patients, and is not transmitted in the community. We then turn to laboratory acquired  
59 infections (LAI) while handling diagnostic or research samples and revealing that most  
60 infections were due to breaches of biosafety procedures in place and that a surprising high  
61 number of these infections had a mild or self limiting course. Finally we look at inactivation  
62 procedures for diagnostic samples to then formulate our recommendations for working with

63 CCHFV.

64

65 **Reported mortality rates and seroprevalences**

66 Observed case fatality rates (CFR) in CCHF vary from 2-30%, and are influenced by efficiency of  
67 diagnosis, cohort size sampled, and speed of clinical intervention [1, 2]. Reported CFR include  
68 25% from South Africa [10], 26% from Kosovo [11] and 15% from Iran and Bulgaria [12,13]. A  
69 structured epidemiological investigation in South Africa revealed that all or most infections in  
70 that country result in clinical disease (Fisher-Hoch et al., 1992). Analysis of ProMED entries on  
71 CCHF from 1998 through 2013 reveals a CFR of 13% among 3,426 cases reported from Turkey,  
72 Russia, Iran, Pakistan, and Afghanistan [2]. In South Russia the CFR has decreased from 12-  
73 16% (1953 -1967) through 1.5-2% (2006-2010) to 3.6-5.1% (2011-2013). This is possibly due to  
74 an increased use of diagnostic kits and awareness of CCHF among medical staff [3].

75 Following a regional epidemic in Turkey in 2003 and subsequent spread, 9787 cases with a  
76 CFR of 4.6% were recorded by the end of 2015, which represents the highest number of cases  
77 on record [14]. Studies in Turkey revealed a seroprevalence of 10%-15% in outbreak regions,  
78 with 88% of infections appearing to be subclinical [15,16]. The disease is often milder in  
79 children than in adults [17]. Additionally, the circulation of CCHFV in endemic regions of  
80 Turkey is supported by serological studies on domestic and wild animals, with antibody  
81 prevalences reflecting the feeding preferences of the *Hyalomma* tick species that transmit the  
82 virus. [18-23].

83 CCHFV strain AP92 has been suggested to be less virulent than other CCHFV strains [24-26]. It  
84 was initially isolated in 1975 [27], from *Rhipicephalus bursa* collected from goats in Greece  
85 and AP92-like sequences have only recently been detected in ticks in Greece, Kosovo and  
86 Albania. A CCHFV AP92 like strain was also described in human cases in Turkey but only

87 causing mild CCHF [24,26]. Recent data indicate a high CCHFV seroprevalence of up to 15% in  
88 some CCHF non-endemic areas of Greece (Kastoria) possibly correlated to CCHFV-AP92  
89 transmission by *R. bursa*. This seems to be confirmed by recent data from Kosovo and Albania  
90 [11,28,29]. The serological and epidemiological data support the initial assessment that  
91 CCHFV AP92 may be less pathogenic however there are no laboratory data to confirm this.  
92 In contrast, after 13 years the CFR in Turkey remains about 5% despite major efforts to  
93 implement protection and prevention measures as well as public health training programmes  
94 and social mobilisation [14,15,26,30].

95

#### 96 **Nosocomial CCHF infections**

97 Nosocomial infections were recorded during the first reported outbreaks of 'Crimean  
98 haemorrhagic fever' in 1944 and 1945, and subsequently in other parts of the former Soviet  
99 Union and neighbouring countries (Hoogstraal, 1979). A more recent detailed literature  
100 review of nosocomial CCHF transmission to HCW listed 44 infections in 494 HCW contacts in  
101 12 countries [4]. Nosocomial infections were reported from South Africa [31-35], Mauretania  
102 [36], Sudan [37], Albania [38,39], Kosovo [40], Bulgaria [41,42], Turkey [43,44], Iran [45-47],  
103 Dubai [48], Pakistan [49,50], India [51], Tajikistan [52], Kazakhstan [53] and Germany [54].

104 Nosocomial transmission often occurs during early illness before CCHF is recognized in the  
105 source patient, or where diagnostic laboratory capability is not available, and is usually  
106 associated with lack of, or improper use of, personal protective equipment (PPE). Once CCHF  
107 is recognized nosocomial infection tends to occur most commonly where source patients  
108 manifest severe disease, probably because they develop the highest viraemias. Recent studies  
109 confirm that when a threshold of  $10^8$  viral genomes per ml of blood is exceeded the disease  
110 progresses to fatal outcome [9,55].



111 In general there is a very low CCHFV seropositivity in HCW dealing with CCHF patients in  
112 Turkey [56,57], and data on infections in HCW in Turkey describe, an up to 33% risk of  
113 infection associated with needlestick injuries, and a 9% risk after contact with body fluids [58].  
114 In Iran serological studies revealed a seroconversion rate of 3.8% in HCW exposed to CCHF  
115 patients. The seroconversion was 9.3% in HCW who had unprotected skin contact with body  
116 fluids and 7.1% in those who suffered percutaneous injuries [59]. A more recent study  
117 covering 9 hospitals which managed 50% of CCHF patients in Turkey from 2002-2014 found 51  
118 HCW exposures by needlestick (62.7%), splashes (23.5%) and unidentified cause (13.7%). Only  
119 25 of these 51 exposures led to laboratory confirmed infections and 4 deaths [60].

120 High compliance to and proper use of PPE can indeed minimize the risk of infection as  
121 documented in a study from the Cumhuriyet University Education and Research Hospital in  
122 Turkey, where 1284 confirmed CCHF patients were treated between 2002 and 2012. The total  
123 seropositivity for CCHFV IgG was only 0.53% in HCW in infectious disease wards which showed  
124 a high compliance to PPE of 100%, 88.6%, and 82.9% for gowns, gloves and masks [61]. This is  
125 supported by another survey of 90 HCWs from 3 hospitals in the endemic regions which found  
126 a low seropositivity rate of 1% [62].

127 Altogether the clinical consensus is that simple barrier nursing and PPE can provide a good  
128 measure of protection to HCW [4]. This is for example the case in the Ankara Ataturk Training  
129 and Research Hospital, where HCWs use contact protection (hand hygiene, gowns and gloves  
130 when needed). N95 masks and goggles are used only when dealing with patients with severe  
131 haemorrhagic symptoms in need of aerosol and droplet producing procedures such as  
132 aspiration and intubation. This pragmatic approach reduces full protection to the most severe  
133 cases from which nosocomial CCHFV transmission is most probable. Over the years four  
134 doctors and three nurses had contact with infected blood and body fluids of CCHF patients,

135 through needlestick injury, skin contact, contact to mucosal surfaces, and probable  
136 aerosolization. All index cases were CCHFV PCR positive. The only HCW who developed  
137 seroconversion intubated an unconscious patient who had suffered a seizure. She was  
138 wearing gloves but no respiratory or eye protection.

139 In another incident one HCW from the Ankara hospital forgot to don goggles when performing  
140 an emergency cardiopulmonary resuscitation (CPR) treatment of a severely ill CCHF patient.  
141 When injecting a drug some blood squirted into her eye, which was immediately washed.  
142 Neither infection nor seroconversion resulted from the incident. Furthermore, no  
143 seroconversion was observed in any of the team performing the CPR without protective N95  
144 masks (Z. Kocak Tufan, Turkey, personal communication).

145

#### 146 **Laboratory acquired infections (LAI) while handling patient samples**

147 Modern diagnostic procedures usually compromise extraction of RNA from blood or other  
148 tissues of patients and the performance of an RT-PCR, plus antibody tests on heat-inactivated  
149 serum [57]. Culture of specimens for isolation of virus is performed less frequently.

150 Eight laboratory infections, one fatal, were recorded in Uganda during early investigations of  
151 'Congo' virus in the 1960s. Where known, exposure of patients to infection occurred during  
152 the handling or processing of infected mice (EAVRI Reports cited in [63]).

153 A laboratory assistant infected himself while preparing plasma from a blood sample of a CCHF  
154 patient by centrifugation in 1986 in a laboratory in Rostov-na-Donu, Russia. The assistant  
155 developed a full-blown CCHF clinical picture including haemorrhages but survived after  
156 prolonged convalescence. A high initial CCHFV LD<sub>50</sub> titer on day 1 and seroconversion were  
157 demonstrated [64].

158 In South Africa, a clinical pathology laboratory technologist in a hospital in Kimberly was found

159 to be seropositive for CCHF in 1986, but the presence of IgG antibody could not be  
160 conclusively linked to an earlier benign illness. The technologist routinely wore a laboratory  
161 coat and disposable gloves and performed all manipulations with blood and serum in class II  
162 cabinets. She used automated haematology and clinical pathology machines. A fatal case of  
163 CCHF occurred in 2006 in a technologist in a clinical pathology laboratory in Vereeniging,  
164 South Africa, who putatively only handled blood samples from a deceased CCHF patient in  
165 order to store them in a freezer. He had signed a procedure protocol which instructed him to  
166 wear a laboratory coat and gloves, but nobody observed him storing the samples. The  
167 technologist reportedly had not tested the samples and it was never determined whether he  
168 had worn gloves, or how he was exposed to infection, but virus isolates from the source  
169 patient and the technologist had identical nucleotide sequences. By the end of 2014 a total of  
170 214 cases of CCHF had been confirmed in South Africa since the first case was recognized in  
171 1981. The diagnostic tests involved the handling of 811 acute phase blood samples at BSL-2 or  
172 3 level with PPE (disposable gown, gloves, laboratory spectacles and N95 masks) without  
173 infections or seroconversions being recorded in the diagnostic laboratory, where the  
174 personnel regularly handle such specimens. The equipment used included class II cabinets,  
175 bench centrifuges, PCR thermocyclers, electrophoresis tanks, gel documentation readers,  
176 ELISA plate washers and readers and fluorescence microscopes. Mouse inoculation and tissue  
177 harvesting were performed in class II cabinets and cages were held in a dedicated room with  
178 Hepa-filtered air supply and exhaust.

179 In contrast, the two laboratory infections reported above, occurred in clinical pathology  
180 laboratories in hospitals where CCHF is infrequently encountered so that an adequate state of  
181 awareness is more difficult to maintain (all information on South Africa; R. Swanepoel,  
182 personal communication).

183 In Turkey, laboratory services issued instructions on the taking and shipment of samples, and  
184 made the information widely available on a web page ([www.thsk.gov.tr](http://www.thsk.gov.tr)). Shipments were  
185 strictly controlled and out of necessity diagnostic assays were performed in BSL-2 laboratories.  
186 Samples had to be handled in class II biosafety cabinets using PPE (lab coat , gloves, goggles  
187 and NP95 mask). [30]. Although a BSL-3 laboratory was finally opened in Ankara in 2012, it is  
188 not used for CCHFV diagnostics. At the time of drafting the present report there had been  
189 9,787 clinical and laboratory confirmed cases of CCHF since 2003, and an estimated 90.000-  
190 100000 samples had been processed under BSL-2 conditions [60]. In some hospitals CCHF  
191 blood samples are handled on the open bench by HCW wearing gloves and N95 masks, but no  
192 goggles. (Z. Kocak Tufan and C. Bulut, Turkey, personal communication). Two case of LAI have  
193 been reported one due to a needlestick while drawing blood and one due to handling a blood  
194 sample without wearing gloves [60].

195

#### 196 **Laboratory acquired infections during research**

197 In an incident in the Rostov-na-Donu laboratory in 1970, one of 4 staff members exposed to  
198 aerosols from a flask containing live virus that disintegrated in a centrifuge fell severely ill and  
199 died. In this instance an underlying chronic hepatocholecystis may have contributed to the  
200 fatal outcome [64].

201 In 1973, at the Institute for Epidemiology, Microbiology and Infectious Disease in Alma Ata  
202 (USSR, now Kazakhstan) a scientist preparing CCHFV antigen from suckling mouse brain using  
203 freon extraction, fell severely ill and seroconverted but recovered. It was concluded that  
204 mixing volatile Freon with the brain suspension may have caused formation of aerosols which  
205 were inhaled. As a consequence work with volatile substances such as freon was required to  
206 be performed in chemical cabinets only [65].

207 In 1981, a virologist died in Cairo, Egypt after mouth-pipetting a culture of an CCHFV isolate he  
208 had brought from Iraq (A. A. El-Sanousi, Egypt, personal communication).

209 At the Institute Pasteur de Dakar two accidents were linked to handling suckling mice  
210 inoculated with a diagnostic sample and a tick pool suspension: in 1998 a technician suffered  
211 a needle stick accident, and in 1993 a staff member in breach of regulations handled cages  
212 with infected mice on an open bench without wearing any mask. They both fell ill, but  
213 experienced mild, self limiting disease. Also in 1993, another technician was exposed to  
214 aerosols while preparing sucrose acetone antigen from infected suckling mouse brain since  
215 not all equipment was held in a laminar flow cabinet or in a BSL-3 laboratory. Again the  
216 disease was self-limiting. A BSL-3 laboratory was built in Dakar in 1999. Henceforth, infected  
217 mouse cages were held in a special laboratory and brain material was treated with beta  
218 propiolactone prior to use as antigen in routine ELISA for IgM/G antibody detection and  
219 immune ascitic fluid production in mice.

220 In 1999 a technician inflicted an abrasion on her hand with a needle during a CCHFV baby  
221 mouse brain passage procedure in the National Center of Infectious and Parasitic Diseases  
222 laboratory in Sofia, in Bulgaria. However, she was vaccinated with the Bulgarian CCHFV  
223 vaccine and presented with benign febrile illness only. In 2010, a Turkish laboratory worker in  
224 a university laboratory inadvertently poured a flask with a 10<sup>th</sup> passage CCHFV culture down  
225 the front of her labcoat but was not infected nor seroconverted (Aykut Ozkul, Turkey,  
226 personal communication).

227

## 228 **Inactivation**

229 Several publications have shown that chaotropic guanidine-isothiocyanate in commercial  
230 nucleic acid extraction buffers efficiently inactivates most enveloped viral agents including

231 pox-, alpha-, bunya-, flavi- and filoviruses [66-68].  
232 Non-treated acute phase serum samples of CCHF patients stored at +4°C remain real time-PCR  
233 positive for up to 30 days but the infectivity of these samples was not verified (A Kubar,  
234 Turkey, personal communication). For serological analysis diagnostic laboratories in Turkey  
235 and South East Europe use thermal inactivation of serum at 56°C for 30 min or even 45 min  
236 although it was concluded in one study that 60°C for 60 min is more effective for CCHFV [69].  
237 In experiments recently performed in the South African laboratory to clearly analyse the  
238 conditions needed to inactivate CCHFV, CCHFV (strain SPU4/81) culture fluid with a titre of  $1 \times$   
239  $10^{7.6}$  TCID<sub>50</sub>/ml was incubated at 56°C and 60°C for 15, 30, 45 and 60 minutes and then  
240 inoculated into Vero E6 cell cultures. In all instances virus growth was not detected. To show  
241 that the results were not due to the detection limit of the TCID assay at  $1 \times 10^{1.5}$  TCID<sub>50</sub>/ml,  
242 the inactivated suspensions were also inoculated intracerebrally into suckling mice (NIH strain)  
243 and all mice survived, even those inoculated with virus inactivated at 56°C for only 15 minutes  
244 (Figure 1). The experiments confirm that heat inactivation at 56°C/30 min used as a standard  
245 in Turkish (national guideline) and many other laboratories in south-eastern Europe is  
246 adequate for destruction of infectivity, and explains why LAI have not been reported from  
247 these diagnostic laboratories.

248

#### 249 **Biosafety regulations**

250 The UN Convention on the Prohibition of the Development, Production and Stockpiling of  
251 Bacteriological (Biological) and Toxin weapons and on their Destruction (BTWC) as  
252 promulgated in 1972 imposed requirements on member states (party to the convention) for  
253 acquiring, holding, stockpiling, working with or disposing of certain pathogens (the list  
254 includes CCHFV) at specified biosafety levels, but BTWC lacked an organization or mechanisms

255 to monitor and enforce compliance. Consequently, UN Security Council Resolution 1540 was  
256 passed in 2004 to enforce domestic compliance on states parties as well as non-state actors  
257 through a 1540 committee. Purely diagnostic procedures and laboratories are exempted. It  
258 should be noted that documents such as the Laboratory Safety Manual [WHO, 2004], the  
259 Biosafety in Microbiological and Biomedical Laboratories manual [56], and the European CEN  
260 Workshop Agreement 15793 [CWA 15793, 2011] only make recommendations on biosafety,  
261 and do not impose legal requirements. Each country must promulgate its own biosafety  
262 legislation and regulations, and many have not yet done so. Consequently, there is wide  
263 divergence in the biosafety levels prescribed for handling CCHFV as some countries attempt to  
264 reconcile disease endemicity with laboratory capacity.

265 In a recent survey of laboratories in 28 countries that are members of the European Network  
266 for Diagnostics of Imported Viral Diseases (ENIVD), it was found that 7 countries sent  
267 diagnostic samples for CCHF to reference centres elsewhere, 5 tested samples in BSL-2  
268 laboratories, 10 in BSL-3 laboratories and 6 in BSL-4 laboratories. Of 11 laboratories  
269 performing virus isolation and propagation, 6 did so in BSL-4 facilities and 5 in lower-grade  
270 facilities [70]. Enquiries made for purposes of the present review revealed that in Slovenia,  
271 Turkey and Senegal CCHFV diagnostic samples were handled at BSL-2 for years before a BSL-3  
272 laboratory was finally available for research. In many other countries including Turkey, Kosovo,  
273 Albania, Bulgaria diagnostics are still performed at BSL-2. Even in the US diagnostic samples  
274 are not handled in BSL-4 but in BSL-2 laboratories until the presence of CCHFV has been  
275 confirmed. In most non endemic countries diagnostic investigations however are conducted in  
276 BSL-4 facilities. All countries tend to use higher grade facilities for research (Table 1).

277

278 **Discussion**

279 In non-endemic countries that coincidentally tend to be better resourced and can afford  
280 sophisticated laboratories, CCHFV is classed and handled as a hazard group 4 agent (Table 1).  
281 Agents in this group cause severe disease, are a serious hazard to staff, are likely to spread to  
282 the community and there is no effective prophylaxis or treatment. In contrast, most endemic  
283 countries have performed diagnostic tests under BSL-2 or 3 conditions, using  
284 appropriate PPE and laboratory practices. In particular, Turkey and several of the Balkan  
285 countries have processed large numbers of specimens without experiencing any LAI over  
286 many years. Although virological and seroepidemiological studies indicate that strains of virus  
287 circulating in the region may have reduced virulence, this alone does not account for the lack  
288 of observed LAI since monitoring for seroconversion confirms that transmission to HCW is rare.  
289 The present survey was performed to collect information on LAI in hospitals, and diagnostic  
290 and research laboratories. Only a few were identified. Such infections as have been reported  
291 in BSL-2 diagnostic laboratories almost invariably result from breaches of biosafety practice.  
292 Handling samples without gloves or mouth pipetting used in the initial isolations of CCHFV in  
293 the 1950s are no longer acceptable. Lessons have been learned from exposure to droplets in  
294 research settings, and in particular centrifuge buckets should be fitted with biosafety seals  
295 (clip on lids), and hazardous procedures should be performed in biosafety cabinets [71].  
296 Outside of cabinets, culture flasks should be carried in sealed boxes, lids should be used on  
297 ELISA and culture plates during incubation, and film seals used for reading of plates. Sera  
298 should be heat-inactivated at 56°C/30 min before performing antibody tests.  
299 Safety can be increased by wearing PPE commonly used in BSL-3 laboratories (face shield  
300 instead of goggles), without necessarily having to rely on positive pressure respirators.  
301 Accidental spillage of infected material unfortunately remains a possibility, but need not  
302 necessarily have a serious outcome as exemplified by the spill onto a Turkish laboratory



303 worker. Animal inoculation procedures should preferably be avoided in diagnostic  
304 laboratories that do not have BSL-3 or -4 facilities. For BSL-3 laboratories measures as  
305 implemented in Dakar are advisable.

306 There is however an ongoing debate on aerosol transmission of CCHFV in clinical settings  
307 There are only few reports describing the infection of close relatives of CCHFV patients, and  
308 these lack conclusive evidence of aerosol transmission [72,73]. On the contrary none of a  
309 cohort of 132 relatives staying with or visiting 88 CCHF patients of whom two patients died at  
310 the Cumhuriyet University Hospital in Turkey, developed any symptoms nor seroconverted  
311 despite the fact that many did not comply with protective measures [74]. The study indicates  
312 that CCHF is not easily spread between humans and into the community.

313 Although multiple-case incidents of nosocomial infection have been reported (Mauretania,  
314 Sudan, Pakistan [4]) there is no evidence for aerosol transmission in CCHF, and spread of  
315 infection was generally postulated to result from direct contact with body fluids or droplets of  
316 severely ill patients, percutaneous injuries and non compliance with basic infection control  
317 precautions.

318 A recent review of possible aerosol (1-5 $\mu$ m) or droplet (5-10 $\mu$ m) transmission through  
319 coughing and vomiting in Ebola virus disease, concludes that there are no epidemiological  
320 data to support a large role for this mode of transmission, and that respiratory transmission  
321 (aerosol generation in the lung, exhalation and transmission by inhalation) does not occur [75],  
322 and the same appears to apply to what is currently known about CCHF transmission. In  
323 contrast aerosol transmission is well documented for smallpox virus and was conclusively  
324 shown by retrospective smoke experiments after isolated patients caused nosocomial  
325 transmission in Meschede in Germany [76].

326 However if actively generated, aerosols are indeed very likely to increase transmission, as

327 recently described in a clinical setting due to the use of a compression inhaler to apply  
328 mucolytics and broncholytics to a CCHF patient while only surgical masks were used by HCW  
329 [77]. In a most recent report 2 HCW suffered an infection probably while using bag-valve-  
330 mask ventilation, or performing bronchoscopies on an infected patient [54]. The obvious  
331 conclusion is to use fitted N95 masks if inhalation devices are used or aerosols might be  
332 actively generated in any other way. On the other hand, care has to be taken when using this  
333 type of masks, as unpublished data from Public Health England (Nigel Silman, UK, personal  
334 communication) show that the filter of N95 masks should not be used for more than 2 hours  
335 as humidity trapped in the mask will bridge the filter, thus negating its efficiency.

336 In conclusion, diagnostic tests have been performed safely at BSL-2 level for many years in  
337 CCHF endemic countries that could not otherwise cope with demand. We therefore  
338 recommend that regulating authorities should revise biosafety requirements for CCHF  
339 diagnostic procedures to allow the required tests to be performed under enhanced BSL-2  
340 conditions with appropriate biosafety laboratory equipment and PPE used according to  
341 standardized protocols in the affected countries (see box 1). In this respect we'd like to point  
342 out that class I cabinets which draw air away from the operator are preferable to class II  
343 cabinets which provide a sterile working area through creating a laminar flow. Organizations  
344 such as the Centres for Disease Control and Prevention, USA, the National Institutes for Health,  
345 USA, the World Health Organization, and the European Committee for Standardization, should  
346 revise international recommendations accordingly. Technical advances arising from the  
347 successful deployment of mobile BSL-3 laboratories in the West African outbreak of Ebola  
348 disease [78-81] should be exploited to derive cost-effective improvements to diagnostic  
349 laboratories in the CCHF endemic countries. In particular, the use of flexible-walled or hard  
350 plastic glove boxes for extraction of nucleic acids and inactivation of sera would greatly

351 improve laboratory safety. The evidence on LAI and LAI outcome, transmissibility and CFRs  
352 should merit to discuss the possibility of relaxing biosafety standards for research on CCHFV,  
353 and the graded application of isolation precautions in the treatment of patients according to  
354 clinical status should be codified.

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### 358 **Acknowledgements**

359 Funding was received through CCH Fever Network (Collaborative Project) supported by the  
360 European Commission under the Health Cooperation Work Program of the 7th Framework  
361 Program (Grant agreement no. 260427) (<http://www.cch-fever.eu/>).

### 362 **Disclaimer**

363 The views expressed by state employed American co-authors are their personal views, and do  
364 not necessarily represent the views of the US government agencies they work for.

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Table1. CCHFV hazard groups and biosafety levels

Country	Endemic	Hazard group	Biosafety level of CCHFV diagnostics	Biosafety level of CCHFV research
South-Africa	+	2	BSL-2 1980-2004 BSL-3 since 2004	BSL 4
Slovenia	-	4	BSL-2 1995-2004 BSL-3 since 2004	BSL 3 since 2004
Albania	+	2	BSL-2	BSL-3
Kosovo	+	2	BSL-2	BSL-2
Greece	+	4	BSL-2 1975-1987	BSL 3 glovebox introduced in 1987
Bulgaria	+	3	BSL-2	BSL-3
Serbia	+	?	BSL-2	----
Turkey	+	2	BSL-2	BSL-3 since 2012
Kazakhstan	+	4	BSL-3	BSL-4
Georgia	+	4	BSL-3	BSL-4
Iran	+	3	BSL-2 glovebox	
Senegal	+	3	BSL-2 glovebox 1990-1999 BSL-3 2000-2015	BSL 3
Germany	-	4	BSL-4	BSL 4
Sweden	-	4	BSL 4	BSL 4
United Kingdom	-	4	BSL 4	BSL 4
France	-	4	BSL 4	BSL 4
United States	-	4	BSL-3	BSL 4

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**Recommendations for working with CCHFV**

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**Primary containment**

BSL-2 laboratory

Class I / Class II biosafety cabinet\*

**PPE**

Labcoat

Gloves

Goggles / Face-shield

N95 mask

**Additional Procedures**

Thermal inactivation of samples at 56°C/30min

Guanidin-thiocyanate based nucleic acid extraction

Seal ELISA plates with transparent film before removing from biosafety cabinet

Use centrifugation buckets with clips on lids, open buckets in biosafety cabinet only.

\*. It is recommended to switch to class I cabinets if possible.

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628 Figure legend

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630 Figure 1. Percent survival of suckling mice i.c. injected with CCHFV-FBS mix ( $1 \times 10^{7.3}$  TCID<sub>50</sub>/ml ).  
631 Dark grey dots: Untreated CCHFV-FBS mix (positive control, n=9 mice). Grey squares CCHFV-FBS  
632 mix treated at RT/15min (n=4), Grey triangles: CCHFV-FBS mix treated at RT/60min (n=9). White  
633 circles: CCHFV-FBS mix treated at 56°C for 15/30/45/60 minutes (n= 8/8/8/5), White triangles:  
634 CCHFV-FBS mix treated at 60°C for 15/30/45/60 minutes (n= 10/8/10/10). Please note that due to  
635 overlay only on line with white circles and one line with white triangles can be seen on the graph.

