Evaluation of SARS-Coronavirus Decontamination Procedures

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Abstract

When the first wave of the SARS epidemic seemed to have reached its conclusion, it was completely unclear how the spread of the virus would evolve. Taking into account all the uncertainties and anticipating the worst-case scenario, many laboratories and vaccine manufacturers started working on a vaccine approach against SARS infection. The results presented here describe the evaluation of decontamination practices performed within the framework of a SARS-Coronavirus (SARS-CoV) vaccine development project.

We show that it takes 45 days at room temperature to fully inactivate the human SARS-CoV, whereas an enveloped virus such as the rabies virus, when treated in a similar way, is totally inactivated in three days. Moreover, the SARS-CoV is very resistant to alkaline treatment and, even more surprisingly, formaldehyde fumigation is not efficacious on the dried virus (under the conditions tested). Only heat (autoclave) and hypochlorite chloride treatments are efficacious treatments for the decontamination of SARS-CoV.

Introduction

In the 1990s, vaccine manufacturers faced a huge increase in the need and demand for vaccines. Existing production processes needed to be optimized to meet the international demands. Amongst the different solutions, the establishment of sequential vaccine production campaigns was an attractive strategy, requiring no additional investment in terms of facilities. However, the issue of cross-contamination between two different viruses handled successively in the same building needed careful attention. The validation of the decontamination of infectious agents became a prerequisite.

One of the first steps was to determine the time necessary for a virus to become inactivated at room temperature. As expected, there are viruses that are relatively resistant, e.g., polio virus (Milstien et al., 1997) and viruses that are very fragile, e.g., rabies virus (Saluzzo & Kusters,

2006). It has been shown that the environment is of extreme importance to the stability of a virus. For example, a viral measles vaccine strain in solution is very sensitive to temperature and light (de Rizzo et al., 1990; Klamm et al., 1991; Sorodoc et al., 1979), but very resistant when the stabilizer (solution of sugar and amino acids), used in the final vaccine formulation, is added (Melnick, 1996). A similar observation was made for influenza virus in cell culture medium solution, which was very sensitive to drying, but more resistant when recovered from allantoic fluid of an embryonated egg (Schaffer et al., 1976). Moreover, these studies show the relative resistance of certain viruses (polio virus, hepatitis A virus) for alkaline treatment (NaOH) (Salo & Cliver, 1976) and the high resistance of polioviruses to detergents (Kawana et al., 1997; Rutala et al., 2000).

The principle lesson learned from these experiments is the extreme diversity of viral characteristics to different decontaminating conditions. Consequently, it is necessary to validate the decontamination conditions for each new virus. In this context, and in reference to the previous published work on viral decontamination practices (Sofer et al., 2003), we present here an evaluation of decontamination practices for the human SARS-CoV.

Materials and Methods

Virus and Cells

The UTAH SARS-Coronavirus was kindly provided by Pat Campbell, from the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, U.S.A. At the CDC, the virus was cloned and passed twice on Vero cells.

Vero cells were grown in flasks to which the virus was added three days later. The harvest was performed three to four days later after infection followed by a 0.8-0.2 μm filtration. The decontamination studies presented here were performed either with crude SARS-CoV infected Vero cell harvest (in culture medium) or on tenfold concentrated harvest diafiltered in PBS buffer. The different studies were performed at room temperature (22°C +:–3°C) with a relative humidity RH of 10-25%.

Determination of Virus Titer

The viral quantification was performed in 96 well plates seeded with Vero cells at a density of 8000 cells per well. Tenfold dilutions of the viral suspension to be titrated were serially performed in Iscove's 4% FCS (Foetal Calf Serum) medium and 200 µl of each dilution was added to the Vero cell monolayers (100 µl per well), with a repetition of six wells per dilution. After three days incubation at 37°C, with 5% CO_2, the cells were examined for cytopathic effects (CPE). The infectious titer was calculated using an in-house method (adapted from Spearman and Kärber) and expressed in $TCID_{50}$ (50% tissue culture infectious dose) units.

Virus Amplification Technique

To ensure the absence of any residual infectious particle after the decontamination treatment, viral amplification testing was performed: 6.25 ml of viral suspension to be tested was placed in a 75 cm² flask seeded with $2x10^6$ Vero cells the day before. The flask was incubated for 7 days at 37°C, with Iscove's medium. The culture medium was removed, the cells were trypsinized and resuspended in a final volume of 500 ml of 4% FCS Iscove medium. The volume was divided into three fractions of 100 ml, each placed in a 175 cm² flask, then incubated at 37°C for seven days. The remaining 200 ml were discarded. One week later, the culture medium was removed, replaced by 1% FCS Iscove's medium and incubated at 37°C for seven days. At the end of the test (D+21), the three flasks were observed for cytopathic effect (CPE). One flask was used for an immunocolorimetry test (in-house test) with a mouse anti-SARS-CoV antibody followed by an anti-mouse antibody coupled to alkaline phosphatase. Antibody binding was revealed with BCIP/NBT substrate kit (BD Biosciences). If necessary, the second flask was used in an immunofluorescence test, based on the same principle but with the second antibody coupled to fluorescein. The final reading was performed with a fluorescent microscope.

Heat Treatment of Viruses

The assay was performed twice with 10-fold concentrated SARS-CoV infected Vero cell culture harvests, at 58°C and 68°C. These two temperatures correspond to the lower limits of 60°C±2°C and 70°C±2°C.

In order to rapidly obtain the right temperature, the viral suspension was diluted tenfold in pre-heated media before being placed in the water bath. After predetermined heating durations, samples were taken from the suspensions and titrated in 96 well plates to determine the infectious titer and establish a kinetic curve. Viral amplification tests were also performed after one hour of heating at 58°C and 68°C, and after two hours heating at 58°C.

Alkaline Treatment of Viruses

The assays were performed with crude harvest SARS-CoV infected Vero cell culture, in duplicate. In the first experiment, the pH of the suspension was adjusted to 11±0.2 and 13±0.2 by NaOH addition. A kinetic study was then performed by sampling the viral suspension after pre-determined treatment durations, neutralizing the pH to 7.0±0.2 with HCl, and determination of the viral titer.

In a second series of assays, the pH was adjusted to theoretical 13 and 13.5 values by adding respectively one volume of NaOH 1N and one volume of NaOH 3N to 9 volumes of the viral suspension. This experimental setting is closer to the real production practices where the pH is not adjusted, but indeed prepared by adding the different volumes of NaOH to the viral suspension. The samples taken for this kinetic study were neutralized to pH 7.0±0.2 with HCl (1N) and the viral titer was determined by titrations on Vero cells.

Drying of Viruses

Viral titer reduction by drying on a surface was evaluated twice by placing 10 ml of crude harvests in 60 mm petri dishes left open in the laboratory in the isolator for pre-determined durations (22°C +/– 3°C; relative humidity 10-25%). Two different drying durations were evaluated. In the first study, the drying lasted six days, whereas in the second study the drying was prolonged for 49 days. After drying, the virus was recovered by swabbing the glass surface and the swab eluted in culture medium (final volume = 10 ml). The suspension was then sampled, frozen at <–70°C, and all titrated on Vero cells in the same titration series to establish a kinetic curve.

In parallel, liquid crude harvest was placed in a closed flask, in the laboratory, at the same temperature conditions as the viral suspension to be dried, to check the eventual viral loss in the liquid form.

The humidity of the laboratory at the time the experiments were performed was around 10-25%.

Gaseous Formaldehyde Treatment of Viruses

Laboratory decontamination is often performed by formaldehyde fumigation. In the experiment presented here, 10 ml of crude harvest was placed in 60 mm petri dishes and left open in the laboratory during the gaseous formaldehyde treatment (22°C +/– 3°C; relative humidity 10-25%; contact time: six hours). After the treatment, the formaldehyde was neutralized by adding sodium metabisulfite to the viral suspension and the neutrality of the pH was checked. The suspension was sampled and frozen at <–70°C before infectious titration on Vero cells. A cytotoxicity control was performed with culture medium formolized and neutralized with sodium metabilsulfite.

To mimic the usual condition of gaseous formaldehyde fumigation, we repeated the tests with dried virus. Another study was performed to try to correlate the mois-

ture of the viral suspension to the formaldehyde fumigation efficiency: viral suspension was placed in petri dishes and submitted to drying for 6 hours, 48 hours and 72 hours, at room temperature (22°C +/– 3°C; residual humidity 10-25%; contact time: six hours), then formaldehyde fumigated and processed as in the first study.

Sodium Hypochlorite Treatment of the Viruses

Sodium hypochlorite (6400 ppm) efficiency was evaluated with crude harvests dried on petri dishes for 24 hours (10 ml of crude harvest in a 60 mm diameter glass petri dish). Five ml of sodium hypochlorite was added to the dried crude harvest and left for one minute, five minutes, or 15 minutes. The surface was swabbed and the swab was discharged in culture medium. The volume was adjusted to the initial volume of crude harvest (10 ml), and 2.5 ml of this suspension was loaded on a PD10 column (desalting columns from GE Healthcare) equilibrated with PBS buffer. After elution with 3.5 ml of PBS, the filtrate was collected, sampled, and frozen at <–70°C. In order to recover the residual particles left on the glass surface after the first washing step, the petri dish was swabbed with 10 ml of culture medium, and the suspension obtained was loaded on a PD10 column. The filtrate obtained (elution with 3.5 ml of PBS buffer) was sampled and frozen at <–70°C.

All the samples were titrated on Vero cells the same day. A control assay was also performed by replacing the sodium hypochlorite solution with PBS in order to ensure that the process ensures satisfactory recovery of virus.

Safety

For safety reasons, all the experiments on SARS-CoV were performed in a laminar air flow cabinet or isolator (except for the gaseous formaldehyde experiments) and all personnel wore double gowns and HEPA filtered positive pressure respirators (PAPR). All the experiments were performed in BSL-3 containment facilities.

Results

Heat Treatment of Viruses

Incubation of SARS-CoV at 58°C or 68°C showed a rapid reduction in infectivity with a mean log viral reduction (LVR) of 4.9 $\log_{10}TCID_{50}$/ml after 30 minutes at 58°C and ≥ 4.3 $\log_{10}$ $TCID_{50}$/ml after 10 minutes heating at 68°C. The results are presented in (Figure 1). Not surprisingly, virus inactivation was more rapid at 68°C than 58°C. The detection limit of the virus titration method (1.5 $\log_{10}TCID_{50}$/ml) was reached after 30 minutes heating at 58°C and 10 to 30 minutes heating at 68°C. Inactivation testing with the more sensitive viral amplification technique (samples heated for 1 hour at 58°C and 68°C) revealed complete inactivation for the samples heated at 68°C. However, residual infectivity was observed with the samples heated for one hour at 58°C, but not in the samples heated for two hours.

Alkaline Treatment of Viruses

The pH adjustment of SARS-CoV suspensions to extreme alkaline values resulted in viral reduction. Alkaline treatment, adjusting the pH of the viral suspensions

Figure 1
Kinetic curve for the heat treatment of SARS-Coronavirus.
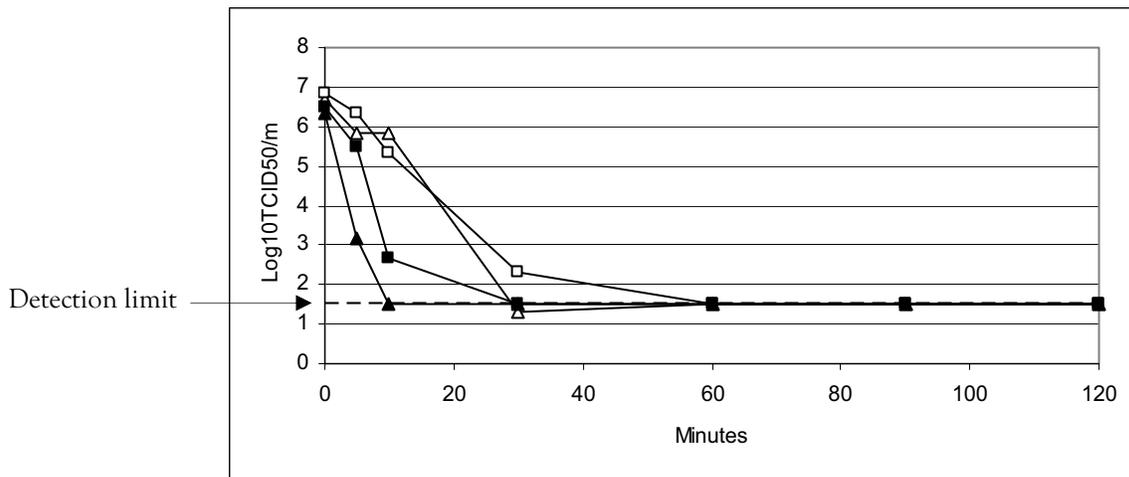
Batch A was heated at 58°C (△) or 68°C (▲) and batch B was heated at 58°C (□) or 68°C (■). The detection limit of the viral titration technique (1.5 log10TCID$_{50}$/ml) is presented with a dotted line and arrows.

to respectively 11 and 13, show that a pH of 11 is not very efficacious for viral inactivation, whereas a pH of 13 inactivates the viral suspension efficiently. The latter effect could be observed immediately after pH adjustment (Figure 2a).

A second series of experiments confirmed the efficient viral inactivation by suspension of extreme pH values of around 13. Indeed, when adding one volume of 1N NaOH to nine volumes of viral suspension followed by homogenization, a mean of 3.6 $\log_{10}TCID_{50}$/ml viral reduction was observed. A stronger concentration of 3N NaOH solution did not seem to have a greater impact, as the viral reduction after adjustment was between 3.63 and 3.98 $\log_{10}TCID_{50}$/ml. After this immediate decrease, the viral titer reduction decreased slowly over time, as shown in Figure 2b. Indeed, after more than six hours of
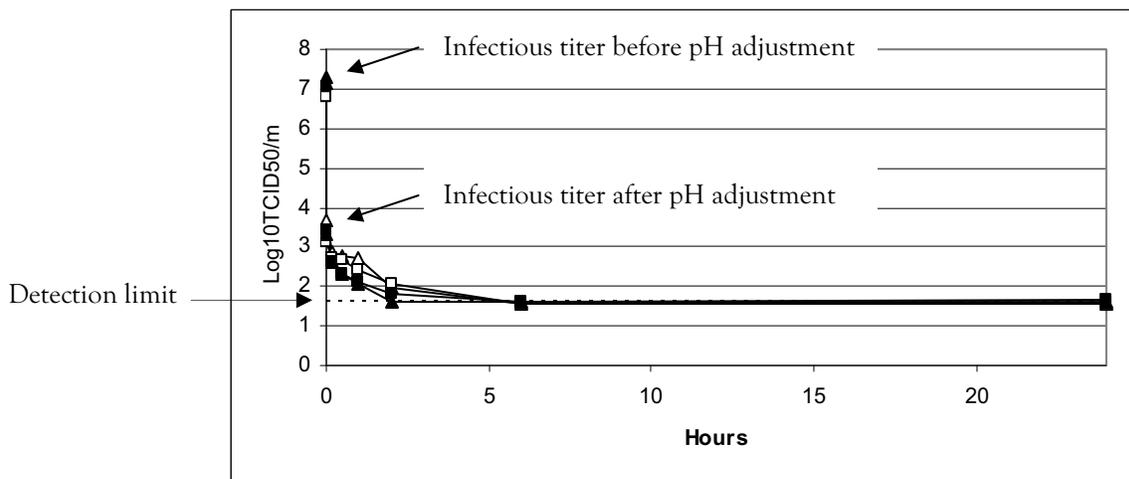
Figure 2a
Kinetic curve for the alkaline pH treatment of SARS-Coronavirus.
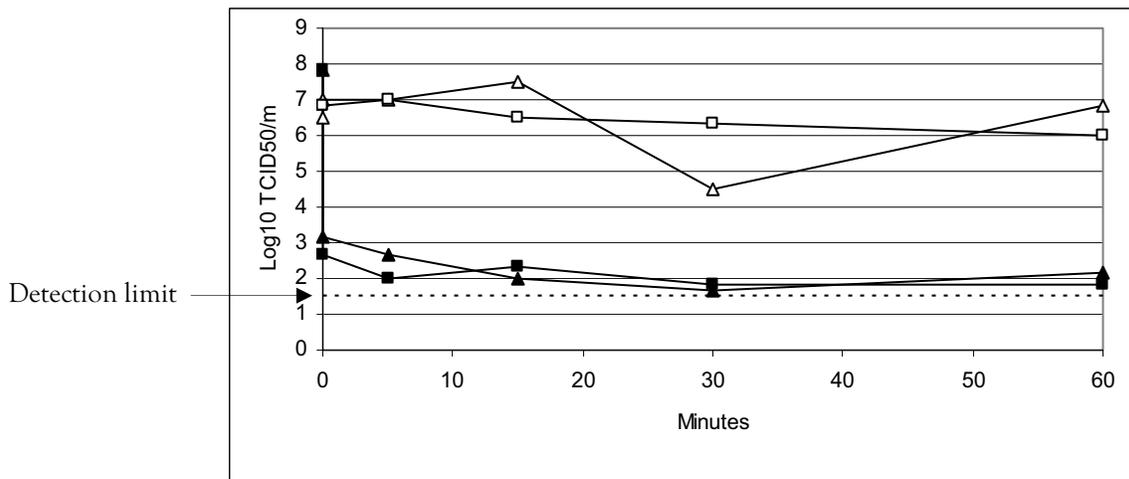
pH was adjusted to 11 for batch A (△) and batch B(□) and to 13 for batch A (▲) and batch B (■). The detection limit of the viral titration technique (1.5 log10TCID$_{50}$/ml) is presented with a dotted line and arrows.

Figure 2b
Kinetic curve for the alkaline pH treatment of SARS-Coronavirus.

9 volumes of batch A viral suspension + 1 volume of 1N NaOH (△) 9 volumes of batch A viral suspension + 1 volume of 3N NaOH (▲) 9 volumes of batch B viral suspension + 1 volume of 1N NaOH (□) 9 volumes of batch B viral suspension + 1 volume of 3N NaOH (■). The detection limit of the viral titration technique (1.5 log10TCID$_{50}$/ml) is presented with a dotted line and arrows.

alkaline treatment, virus was still detectable.

To ensure cytotoxicity was not due to the alkaline pH treatment, a control sample was performed with buffer adjusted to the target alkaline pH and neutralized to pH 7.0±0.2 with HCl (1N). The cytotoxicity of this sample towards the Vero cells was evaluated with the same infectious titration method. No CPE was observed ensuring the absence of cytotoxicity.

Drying of Viruses

Inactivation of SARS-CoV by drying was studied by depositing the virus on a glass petri dish over a period of six days at room temperature. The results obtained showed a maximum viral reduction of 1.59 $\log_{10}$ $TCID_{50}$/ml after six days of drying. Control samples, virus in liquid form, were titrated in parallel. After four days, the liquid samples had lower viral titers than their dried counterparts. The
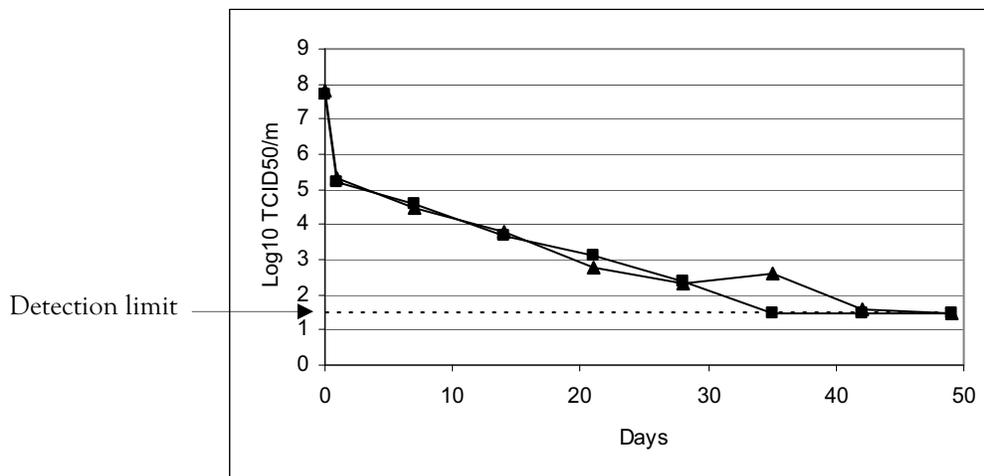
Figure 3a

Kinetic curve for the drying treatment of SARS-Coronavirus over a time period of six days.
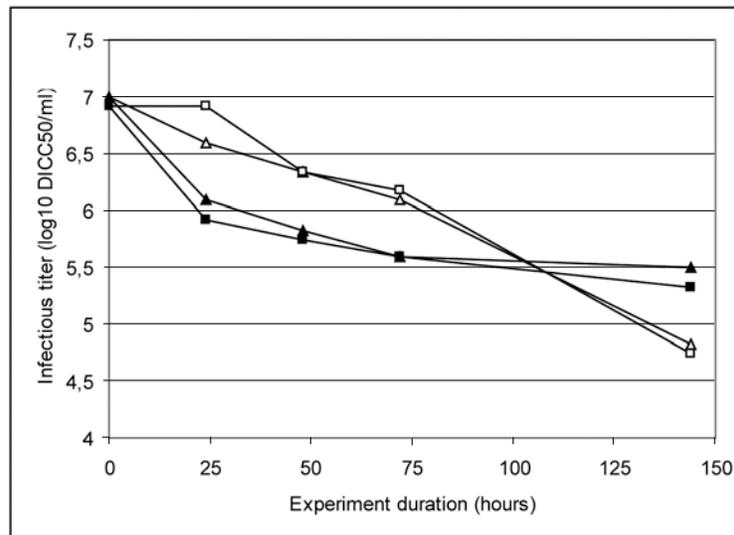
Batch A liquid form (△) and batch B liquid form (□) - batch A dried form (▲) and batch B dried form (■). The detection limit of the viral titration technique is 1.5 $\log 10TCID_{50}$/ml.

Figure 3b

Kinetic curve for the drying of SARS-Coronavirus over a time period of 49 days.

Batch A 49-day experiment (▲) Batch B 49-day experiment (■). The detection limit of the viral titration technique (1.5 $\log 10TCID_{50}$/ml) is presented with a dotted line and arrows.

SARS-CoV seems to be protected under dried conditions, keeping its infectious capacities (Figure 3a).

A kinetic evaluation over a period of 49 days showed the high resistance of the SARS-CoV when dried on a glass surface (Figure 3b). The viral titer was reduced by 2.5 $\log_{10}$ $TCID_{50}$/ml in the first day that is when it passes from the liquid form to the dried form. Then a mean viral reduction of 0.07-0.14 $\log_{10}$ $TCID_{50}$/ml was observed for each day of drying. Altogether, 35 to 42 days were necessary to reach a level where no infectious particles could be detected by the virus titration technique.

Gaseous Formaldehyde Treatment of Viruses

Due to the cytotoxicity of formaldehyde and sodium metabisulfite, the first two dilutions in the 96 well plates were not evaluated and the detection limit of the technique was raised to 3.5 $\log_{10}$ $TCID_{50}$/ml.

For the SARS-CoV in liquid form, we found that the LVR after formaldehyde fumigation in liquid was greater than 3.2 $\log_{10}$ $TCID_{50}$/ml after 72 hours. However, with the dried sample, the fumigation was not so efficacious, as the LVR was 1 $\log_{10}$ $TCID_{50}$/ml for batch A and 1.66 $\log_{10}$ $TCID_{50}$/ml for batch B after 72 hours (Table 1). Therefore, we can state that formol fumigation is not an efficient viral decontamination technique for dried virus at low relative humidity.

Studies on the drying duration and the resistance to formaldehyde fumigation showed that the longer the drying duration the more resistant the virus becomes to formaldehyde fumigation. Indeed, after six hours of drying the mean LVR after formolization was 3.25 $\log_{10}$ $TCID_{50}$/ml and after 48 hours drying, the LVR after formolization was 2.04 $\log_{10}$ $TCID_{50}$/ml. Finally, the sample dried for 72 hours and which was then formolized had a mean LVR of 1.86 $\log_{10}$ $TCID_{50}$/ml (Figure 4).

Sodium Hypochlorite Treatment of the Viruses

Sodium hypochlorite is a strong and often used reagent to inactivate viruses (Sofer et al., 2003) which caused cell toxicity. We solved this problem of evaluating sodium hypochlorite treated material by using PD10 desalting columns and demonstrated that we recovered almost all the loaded viral particles (the LVR for this step was 0.705 $\log_{10}TCID_{50}$/ml for the first wash and 0.83 $\log_{10}TCID_{50}$/ml for the second swabbing step).

The infectious titers obtained with the control sample (no sodium hypochlorite added) after these two steps were 6.05 $\log_{10}TCID_{50}$/ml for the first wash and 4.23 $\log_{10}TCID_{50}$/ml for the following swab, showing clearly that the mechanical action performed with the first washing was not sufficient to remove all the viral particles dried on the glass surface. This reinforced the necessity for a chemical treatment of the surface.

With sodium hypochlorite (6400 ppm) treatment, whatever the duration of the treatment was, we did not detect any infectious viral particle in the solution eluted on desalting PD10 columns. This treatment was very efficient, as after one minute incubation with sodium hypochlorite the LVR was greater than 4.7 $\log_{10}TCID_{50}$/ml for the first wash. After this washing step, no more viral particles were detected on the glass surface, as checked with the second step (swabbing with culture medium) (Table 2).
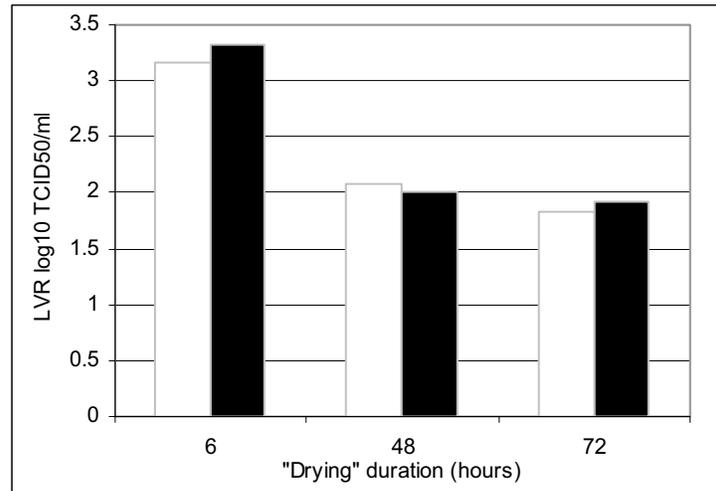
Table 1

Inactivation of SARS-Coronavirus by gaseous formol.

	Batch A				Batch B			
	Infectious titer ($\log_{10}TCID50$/ml)		Log viral reduction ($\log_{10}TCID50$/ml)		Infectious titer ($\log_{10}TCID50$/ml)		Log viral reduction ($\log_{10}TCID50$/ml)	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
Crude harvest liquid form not formolized T0	7.33	7.8	ND	ND	7.00	6.9	ND	ND
Crude harvest liquid form not formolized maintained 72 hours at room temperature	6.67	7.5	0.66	0.3	6.67	6.75	0.33	0.15
Crude harvest liquid formolized	< 3.5	< 3.5	> 3.17	> 4.3	< 3.5	< 3.5	> 3.17	> 3.4
Dried crude harvest not formolized T0	5.67	ND	1.66	ND	6.33	ND	0.67	ND
Dried crude harvest formolized	4.67	ND	1	ND	4.67	ND	1.66	ND

For the dried crude harvest, the LVR is calculated by comparison between the dried virus formolized and the dried virus not formolized. (ND = no data; T0 is Time 0).

Figure 4
LVR obtained after drying and formolization.

From left to right, for each treatment duration (hours): LVR due to drying and formolization, batch A (white bar) - LVR due to drying and formolization, batch B (black bar).

Table 2
Inactivation of SARS-Coronavirus dried crude harvest by sodium hypochlorite (600 ppm) treatment.

	Control sample in culture medium	hypochlorite treatment for 1 minute		hypochlorite treatment for 5 minutes		hypochlorite treatment for 15 minutes	
	Infectious titer ($\log_{10}$ TCID50/ml)	Infectious titer ($\log_{10}$ TCID50/ml)	LVR ($\log_{10}$ TCID50/ml)	Infectious titer ($\log_{10}$ TCID50/ml)	LVR ($\log_{10}$ TCID50/ml)	Infectious titer ($\log_{10}$ TCID50/ml)	LVR ($\log_{10}$ TCID50/ml)
S1 eluted on PD10 column	5.20	≤ 0.50	[3] 4.70	≤ 0.50	[3] 4.70	≤ 0.50	[3] 4.70
S2 eluted on PD10 column	≤ 3.15	≤ 0.50	[3] 2.65	≤ 0.50	[3] 2.65	≤ 0.50	[3] 2.65

Discussion

In March 2003, the World Health Organization (WHO) issued a global alert for the illness that would become known as "Severe Acute Respiratory Syndrome" (SARS) (WHO, 2006b). A few months later, many laboratories and vaccine manufacturers started working on a vaccine against SARS infection. As early as April 25, 2003, the WHO and the CDC in the U.S. (CDC, 2006; WHO, 2006a) recommended that laboratories and vaccine manufacturers handle SARS-CoV specimens using Biosafety Level (BSL-3) work practices. When we started to work with the SARS-CoV in our BSL-3 facilities, in August 2003, there was no data available on the inactivation characteristics of the virus. One of the first

steps and major priorities was to identify the conditions to fully inactivate the virus, for the decontamination of equipment, facilities, and waste. In this paper, we will discuss our findings on the SARS-CoV inactivation characteristics in the context of similar studies on other viruses such as polio, rabies and influenza viruses previously performed in our facilities.

The SARS-CoV can be inactivated by several methods, based either on physical mechanisms or on chemical mechanisms. We investigated five decontamination methods that are currently used for equipment, facilities and waste decontamination: heat, alkaline and sodium hypochlorite treatment, gaseous formaldehyde fumigation and drying. We want to stress that the data presented here has been obtained under our specific experimental condi-

tions. The virus sensitivity to inactivation is dependant on the virus environment and concentration. Thus, the methods presented here were validated with our specific suspensions and experimental conditions and may not apply to SARS-CoV under other laboratory conditions.

Thermal decontamination of human SARS-CoV was shown to be efficient at 58°C and 68°C. As expected, the treatment time necessary to obtain complete viral inactivation decreases when the temperature is increased. One hour of heating was necessary to achieve total inactivation of the SARS-CoV at 68°C and twice this time was necessary at 58°C. Thermal inactivation conditions vary for different viruses. For example, thermal inactivation of influenza virus, as well as poliovirus, does not differ much compared to the SARS-CoV (Lu et al., 2003). These data show that categorization of viruses regarding their heat stability cannot be made in terms of virus families, RNA, enveloped or not, and that for each virus the right inactivation conditions need to be determined. From the literature, there is a general consensus that thermal inactivation (60°C for almost one hour) is a very efficient technique to eliminate viruses.

Our inactivation experiments using different pH values gave rise to unexpected results. When the pH was adjusted to 11 by adding sodium hydroxide, the SARS-CoV was not affected. For the other alkaline conditions tested (pH=13 and pH=13.5), we obtained a biphasic kinetic curve with immediate viral inactivation of 3.5 log viral reduction, followed by a low decrease of infectious titer. Using a very sensitive viral amplification technique, we could still observe infectious particles after six hours of treatment, but no infectious particles were observed after 24 hours.

The long time needed for SARS-CoV inactivation by alkaline treatment is rather unexpected, as usually enveloped viruses are very sensitive to such drastic conditions. For example, for rabies virus, a pH=12 alkaline treatment for 15 minutes was sufficient to obtain a reduction of 6.8 $\log_{10}TCID_{50}$/ml (in-house data) (Saluzzo & Kusters, 2006).

The results from the experiments performed to evaluate the viral loss of the SARS-CoV due to drying on glass surface were also surprising: 35 to 42 days were necessary to inactivate the virus to the detection limit of the technique. This duration is much longer, compared to the data presented in other publications (Rabenau et al., 2005). These differences in findings might be explained by the differences in experimental conditions. In our experience, the SARS-CoV appears indeed to be one of the most resistant viruses to inactivation by drying at the room temperature. For rabies virus, another enveloped virus, it takes only three days to reach complete inactivation. For poliovirus, a non-enveloped and more resistant virus, it takes about a week to reach complete inactivation (Saluzzo & Kusters, 2006).

Gaseous formol fumigation is a viral decontamination technique widely used. In the present study, we confirm the efficacy of this treatment on virus in solution. However, we also studied inactivation by formaldehyde fumigation on dried virus to mimic laboratory situations where a drop of virus can be spilled unintentionally on a surface (bench, floor). These experiments showed that gaseous formol fumigation is not efficient on dried virus. The viral loss is only 1 to 1.6 log after 72 hours of fumigation. From these data, we can conclude that the moisture state of the viral suspension impacts the gaseous formol fumigation efficiency. There is a reverse correlation between dryness and formal fumigation treatment for viral inactivation. Similarly, bacterial spores on nonporous surface are more readily killed at higher relative humidity (Spiner & Hoffman, 1960). We can speculate that the residual humidity condition was not optimal when we performed the study, but it is noteworthy that similar studies performed on dry polio or flu viruses treated by formaldehyde fumigation were successful in inactivating the dry viruses. We need to perform complementary experiments, particularly in varying the residual humidity to confirm the current data on the SARS-CoV stability. However, from our experience, the stability of the SARS-CoV after total drying is exceptional in comparison to other viruses tested in similar conditions (Saluzzo & Kusters, 2006).

The two decontamination procedure evaluations tested here, drying and formaldehyde fumigation, reinforce the necessity to decontaminate the working areas in the laboratory, as well as the equipment, very frequently in order to avoid any drying of viral suspension onto glass or any other kind of surface.

Finally, sodium hypochlorite treatment was evaluated. The effect of sodium hypochlorite on dried virus is very rapid and very efficient, as no infective viral particle was recovered after washing the surface with sodium hypochlorite. Hypochloride treatment is also a very efficacious inactivation method for polioviruses (Saluzzo & Kusters, 2006).

Based upon the data presented here, we recommend the following decontamination strategy: all the solid waste was autoclaved (at 126°C for 120 minutes) as well as the equipment that could resist such a drastic treatment. For liquid waste, the solutions were submitted to alkaline pH treatment for at least two hours and then transferred into a tank for thermal decontamination at 105°C for 30 minutes. The laboratory facilities, and the equipment that could not be autoclaved, were decontaminated with sodium hypochlorite (6400 ppm) solutions before being fumigated with gaseous formaldehyde. Both products, sodium hypochloride and formaldehyde, are toxic for human health and need, of course, to be used according to safety rules.

In our hands, the human SARS-Coronavirus is the most resistant enveloped virus ever described. It takes 35 to 42 days to inactivate the virus by drying and formalde-

hyde fumigation has no effect on the dried virus. This means that existing decontamination strategies cannot be extrapolated to emerging viruses and that the conditions for decontamination should be determined specifically for each new virus.

Acknowledgements

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