SARS virus Detection and Survival in Food and Water:

Setting a research agenda

A World Health Organization (WHO) Workshop
in collaboration with the
Food and Agriculture Organization of the United Nations (FAO) and
Agencia Española de Seguridad Alimentaria

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SUMMARY
At a meeting in Madrid, 8 – 9 May, 2003, the World Health Organization (WHO), in collaboration with the Food and Agriculture Organization of the United Nations (FAO), brought together a group of concerned scientific experts in virus detection and survival in food and water. The purpose was to gain a better understanding of how the SARS coronavirus (SARS CoV) survives in the environment, with particular reference to food, water, faeces and sewage. As a result, the group will work together in a research network, as part of the international effort to coordinate our collective understanding of the science of SARS and prevent it from becoming endemic. The proposed agenda for research covers standardized methods for isolation and quantification of the virus in the environment. Recommendations were also made for relevant studies on the resistance, persistence and inactivation of the virus under conditions commonly found in food processing and water treatment, as well as sanitation and sewage treatments. Investigations related to faecal-oral transmission would be precautionary in nature since there is no evidence or epidemiological indication that the virus can be transmitted through this route. Nevertheless the research network is looking into potential future scenarios and the research needs that would follow.

1 BACKGROUND
Investigations of the global outbreak of SARS have shown that the major mode of transmission of the SARS virus is through close person-to-person contact, in particular exposure to droplets of respiratory secretions from an infected person. However, in consideration of the fact that WHO believes there is a window of opportunity to prevent this disease from becoming endemic, and that there are positive indications from Viet Nam, Canada, Hong Kong and Singapore that this may be possible, the need for a broad research agenda is emphasized. Part of such a broad research agenda would be the search for a better understanding of how the SARS virus survives in the environment, including in food and water. Although contaminated food and water have not been identified as risk factors for SARS, WHO feels it is prudent to define a potential research agenda in this area, even if only to rule out any concerns.

The need for a broad research agenda is underlined by the findings in a cluster of SARS cases in an apartment block in Hong Kong, where sewage is believed to have played a role through the discharge of droplets containing SARS CoV from the sewage system. WHO sees the need for research to elucidate any potential modes of transmission of the SARS virus through sewage, faeces, food and water. The potential for infection by ingestion, in addition to the close person contact route, must also be considered. However, as stated on 11 April 2003, WHO does not at present conclude that any goods, products or animals arriving from SARS-affected areas pose a risk to public health.

Therefore, WHO in collaboration with FAO and the Spanish Food Safety Agency (Agencia Española de Seguridad Alimentaria) organized a workshop on SARS virus detection and survival in food and water. The Workshop was held in Madrid from 8 - 9 May 2003.

The workshop in Madrid was held in the context of WHO's overall endeavour to improve our understanding of this new virus. Several studies on its survival in the environment have already been undertaken, many of them linked to the WHO network of collaborating laboratories[1]. Scientists in the WHO network have recently reported results of the first scientific studies designed to determine the survival time of the SARS virus in different environmental media. Theses recent studies were primarily conducted at network laboratories in Hong Kong, Japan, and Germany.

[1]A network of 11 laboratories in 9 different countries was established by WHO on 17 March, 2003 to search for the causative agent of SARS

The outcome of the workshop presented in this report is intended to be advice to WHO and other international agencies on the setting of a potential research agenda related to detection and survival of SARS virus in food, water and sewage.

2 SARS – THE CURRENT SITUATION

2.1 Overview of the Global Epidemiology of SARS

Severe Acute Respiratory Syndrome (SARS) is a newly recognized form of atypical pneumonia that emerged in Guangdong Province China in November, 2002. It was not until February 2003, when a medical doctor from Guangdong journeyed to Hong Kong while suffering from the infection and transmitted the virus to several foreign travellers, that a global epidemic began. WHO issued a global alert concerning SARS on 12 March after cases were reported in Hanoi, Viet Nam, Singapore and Toronto, Canada. As of 8 May, over 7 000 probable cases of SARS have been reported from 31 different countries, including 506 deaths. Areas with recent local transmission include several parts of China including Hong Kong and Taipei, Taiwan; Toronto, Canada; Philippines and Singapore. While the majority of cases have been among health care workers and their close contacts, suggesting person to person spread through contaminated droplets, there have been some unusual circumstances for transmission. These include two clusters in Hong Kong; the Hotel M., where the index case from Guangdong infected foreign travellers, and the Amoy Gardens apartment complex where over 300 people became ill. In the latter environment, contaminated sewage is believed to have played a role. A WHO team is currently in the field working with Hong Kong officials to better understand the factors that led to a cluster of SARS concentrated in one apartment block. The SARS outbreak has shown us that in the world today, infectious diseases in one country can be a threat to global health. International collaboration and prompt local action have been key to containing the spread of SARS.

2.2 Laboratory: state of knowledge

A network of 11 laboratories in 9 different countries was established by WHO on 17 March, 2003, to search for the causative agent of SARS. By the end of the 3rd week in March, a coronavirus had been detected and isolated. A monkey model was used to test Koch's postulates for causality confirming this coronavirus is the causative agent of SARS in humans. The virus was consistently found among probable cases, while no other pathogen was consistently associated. DNA sequencing and phylogenetic analysis of the SARS CoV show this virus does not fit into any previously recognized groups of *Coronaviridae*. Several different primer sets have been developed for virus detection and are being evaluated for their sensitivity and specificity. Studies into the kinetics of virus excretion have shown that in severe cases excretion of the virus in stool and sputum may exceed 33 days. In the early stages of disease, virus is not systematically detectable before day 3 of illness. Transmission appears to be through close person to person contact via infected droplets, aerosols or direct contact with infected secretions. The role of faecal-oral transmission remains unclear. The virus appears to be more stable in the environment than known human coronaviruses. For example, the virus can survive after drying on plastic surfaces for 48 hours. For these reasons, strict biosafety procedures are recommended in the laboratory. The biosafety level will vary depending on the nature of work being performed. See WHO SARS's web site for recommendations on biosafety levels and handling procedures (http://www.who.int/csr/sars/en/index.html).

3 CORONAVIRUSES:

SARS CoV belongs to the genus *Coronavirus* within the family *Coronaviridae* but differs from the three known antigenic groups within the genus. A second genus within the family *Coronaviridae* is the genus *Torovirus*. The Coronaviridae belong to the order of the *Nidovirales* together with Arteriviruses and Roniviruses. (The Universal virus database, International Committee on taxonomy of Viruses; http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/index.htm). In this document, we refer to the members of the genus *Coronavirus* as "coronaviruses". Coronaviruses are enveloped, positive, single strand RNA viruses. Coronaviruses are known to be the causative agent of a number of animal and human diseases (Table 3.1). The genome of the SARS CoV is closely related to that of coronaviruses but it does not belong to any known CoV groups. Coronaviruses usually have a narrow host range.

Table 3.1: Coronavirus induced diseases.

Coronavirus induced diseases			
Group	Species	Prototypes	Disease
1	Human	HCoV-229E	Respiratory
	Porcine	TGEV	Enteritis
		PRCV	Respiratory
		PEDV	Enteritis
	Canine	CCoV	Enteritis
	Feline	FCoV	Peritonitis, enteritis
2	Human	HCoV-OC43	Respiratory
	Bovine	BCoV	Enteritis
	Murine	MHV	Hepatitis
		JHM	Neurodemyelination
		RtCoV	Respiratory
		SDAV	Adenitis
3	Avian	IBV	Respiratory
		TCoV-I	Nephritis
Unclassified	Lapine	RbCoV	Cardiomyopathy
	Human	HCoV-DAL-1	Necrotizing enterocolitis (?)

3.1 Coronaviruses: survival outside the host and resistance to environmental factors:

The persistence of viruses in the environment depends on a series of physical factors (temperature, light, desiccation, hydrostatic pressure), chemical factors (pH, salinity, presence of inorganic ions and organic molecules), and biological factors such as the type of virus. Coronaviruses are resistant to a wide range of pH values and have been found to remain infectious for up to 1 year at pH ranges from 2 to 12 (Table 3.2). They have also been found to be resistant to low temperatures and remain active at both refrigeration temperature (4C) and freezing temperatures (-70C) as well as freeze-thawing processes. Information about their behaviour at freezing temperatures commonly used in food processing (-18C-20C) are scarce. However, some enveloped viruses are less stable at -20C (more common for food storage) than at -70C and because of their envelope, they are found to be

labile to common detergents. Little is known about their resistance to high temperatures, UV-radiations or other environmental conditions found in food and water processes. Similarly, little is known about possible mode of coronavirus contamination of the food supply or water. Table 3.2 summarizes some of the conditions influencing coronaviruses. For comparison, Table 3.3 summarizes data on inactivation of other foodborne viruses.

Table 3.2: Inactivation of coronaviruses. Based on Koopmans & Duizer (2002)

Method	Virus (ref)	Inactivation	Effective[1]	Remarks
Thermal treatment				
- 80°C, tissue culture supernatant (TCS)	**SARS (WHO website)**	*Survival > 21 days (starting dose 10^6, no quantitative data)*		
4°C, TCS	**SARS (WHO website)**	*Survival > 21 days (starting dose 10^6, no quantitative data)*		Astrovirus & HAV: 2D in 25 days
22 ^{0}C, 8 days, TCS	HCV 229E (Lamarre &Talbot, 1989)	3 D[2]	No	Astrovirus & HAV: 2D in >50 days
22°C, virus in stool	*SARS (WHO website)*	*pH 6: >3D, 3 hrs* *pH 8: > 3D, 6 hrs* *pH 9: > 3D, 4 days*		
22°C, TCS, 1 % FCS, 2 days	*SARS (WHO website)*	*1D*	**No**	
37 ^{0}C, 3 days, TCS	HCV 229E (Lamarre &Talbot, 1989)	3 D	No	
56 ^{0}C, 30 min, TCS	RCV (Gaertner, 1993)	complete	Yes	
56 ^{0}C, 15 min, TCS	SARS (WHO website)	4D	Yes	
25 ^{0}C, 24 h, dried	RCV (Gaertner, 1993)	~3 D	No	
60 ^{0}C, 1 min, TCS	MHV (Saknimit, 1988)	~3 D	No	
60 ^{0}C, 5 min, TCS	MHV (Saknimit, 1988)	> 4D	No	
60 ^{0}C, 1 min, TCS	CCV (Saknimit, 1988)	~1.5 D	No	
60 ^{0}C, 5 min, TCS	CCV (Saknimit, 1988)	~2.5 D	No	
60 ^{0}C, 15 min, TCS	CCV (Saknimit, 1988)	> 4D	Yes	
80 ^{0}C, 1 min, TCS	CCV (Saknimit, 1988)	> 4D	Yes	
PH				
				No data for SARS. Acid sensitivity important in determining risk of oral infection
4 ^{0}C, 6 h, TCS, 4<pH<9	HCV 229E (Lamarre &Talbot, 1989)	<1 D	No	
4 ^{0}C, 6 h, TCS, pH 3 & pH 11	HCV 229E (Lamarre &Talbot, 1989)	>4 D	Yes	

33 ^{0}C, 6 h, TCS, 5<pH<8	HCV 229E (Lamarre &Talbot, 1989)	<1 D	No	
33 ^{0}C, 6 h, TCS, pH 4 & pH 9	HCV 229E (Lamarre &Talbot, 1989)	>4 D	Yes	
Disinfection				
0.24% chloroxynol	HCV OC43 (Wood&payne, 1998)	<1 D	No	
0.2% Benzalkoniumchloride	HCV OC43 (Wood&payne, 1998)	< 1D	No	
0.1% Cetrimide + 0.01% Chlorhexidine	HCV OC43 (Wood&payne, 1998)	< 1D	No	
70% Ethanol, 10 min, RT	CCV (Saknimit, 1988)	> 3.3 D	Yes	
70% Ethanol, 10 min, RT	MHV (Saknimit, 1988)	> 4 D	Yes	
100 ppm sodium hypochlorite	CCV (Saknimit, 1988)	~1 D	No	
100 ppm sodium hypochlorite	MHV (Saknimit, 1988)	~2.5 D	No	
Survival on food/water				
- 25°C, 30 days	Meat from TGEV-infected pigs (Forman, 1991)	Not known, but infection in all pigs fed ground meat		
?	Meat from clinically healthy TGEV infected pigs (Cook, 1991)	Infection in all experimental pigs fed meat		
Other potential modes of contamination				
Experimentally exposed flies	TGEV infected pigs (Gouch, 1983)	Recovery of infectious virus from flies after 72 hours		
Slurry	TGEV infected sows (Derbyshire, 1978)	Recovery of infectious virus		

[1] effective, i.e.: more than 4 logs inactivation, and practical

[2] D = $\log_{10}$

Table 3.3: Food processes, virus inactivation factors and resulting risk of the product if viruses are present pre-processing**.** Based on Koopmans & Duizer (2004) and Koopmanns & Duizer (2002).

Viruses included in this table are the foodborne Hepatitis A virus (HAV), Norwalk like viruses (NLV) (and the animal model viruses Feline calicivirus [FeCV] and canine calicivirus [CaCV]), Human Rotavirus (HRV), Rhesus Rotavirus (RV), and Poliovirus (PV). Note: the estimates included in this table are based on extrapolation of data from scientific studies, and should be regarded as indicative only. For precise process calculations or predictions on food manufacturing processes, additional experimental information is needed.

Process	**Example of food product**	**Virus inactivation (log10)**	**Risk to public health if viruses are present pre-processing** [1]	**Likelihood of presence pre-processing**[2]	**Remarks**
Thermal Treatments					
Boiling at 100 °C	Any liquid food (e.g. milk) or solid food boiled in water.	HAV and PV > 4	Negligible	Unlikely	Likelihood of presence depending on food. Kinetic data lacking.
60 °C, 30 min (liquids or solid foods)		HAV< 2 [18] or HAV > 4 [11,30] PV < 2 [31] NLV: incomplete [13]	Medium		Inactivation in solid foods lower than in liquids; dependent on fat and protein content.
Pasteurization of solid foods (70°C or equivalent, 2 min)	Pate and other cooked meats.	HAV < 2 [29] FeCV > 3 [14]	Medium	Unlikely	Inactivation dependent on fat and protein content
Pasteurization of liquids and immediate packing (e.g. HTST 71.7°C for 15 sec)	Milk, Ice cream. .	HAV < 2 [4]	Medium	Possible	Inactivation dependent on fat and protein content
UHT & aseptic filling (> 120 °C)	Long life milk, other dairy products.		Negligible	Unlikely	
Other physical/chemical/biological processes					
Commercial drying (spray & freeze drying)	Dried milk, instant dried soups, dessert mixes, chocolate	HAV, FeCV<1 [14, 26]	High	Unlikely	No information on commercial drying
Freezing	Ice-cream, frozen desserts.	HAV, PV, FeCV < 1	High	Possible	
Fermentation	Cheese, yoghurt Ppvc is unlikely.	No information		Unlikely	Microbial inactivation of viruses is found for sludge [22]

Acidification	Fruit juices, still fruit drinks.	NLV: pH 2.7, 3h incomplete [13] HAV: pH 1, 5h incomplete [18]	Medium	Possible	No quantitative data on inactivation
Homogenization		Incomplete	High		Likelihood of presence depending on type of product
Depuration of oysters and mussels		NLV Incomplete [16]	High	Likely	
High hydrostatic pressure (600 MPa, 1h)		PV < 1 [44]	High		Likelihood of presence depending on type of product

[1] ***Negligible risk:*** product highly unlikely to contain infectious viruses; treatment results in at least 4 $\log_{10}$ inactivation of common foodborne viruses.
Low risk: product unlikely to contain infectious viruses in numbers likely to cause disease in healthy individuals: treatment results in approximately 3 $\log_{10}$ inactivation of common foodborne viruses.
Medium risk: product may contain infectious viruses in numbers which may cause disease; treatment results in approximately 2 $\log_{10}$ inactivation of common foodborne viruses.
High risk: products where the level of viruses is likely to be high enough to cause disease in healthy individuals: treatment results in less than 1 $\log_{10}$ inactivation of common foodborne viruses.
Variable risk: treatment results in significant differences in inactivation of several common foodborne viruses

[2] ***Unlikely*****:** no reports are known in which NLV, HAV, RV or PV were found on the food items mentioned**.**
Possible**:** Sporadic contamination with NLV, HAV, RV or PV of the food items mentioned is reported**.**
Likely**:** Contamination with NLV, HAV, RV or PV of the food items mentioned is reported frequently.

Coronaviruses appear to be more resistant at lower temperatures close to refrigeration temperatures compared to ambient temperatures as shown in Table 3.4.

Table 3.4: Survival of human coronavirus HCV229E (after Ijaz et al., 1985)

Temperature	Humidity	Recovery	Half-time
19-21°C	25-35%	85-90%	21-33 h
	45-55%	89-93%	59-76 h
	75-85%	52-59%	~3 h
5-7°C	25-35%	88-94%	31-38 h
	45-55%	94-100%	93-112 h
	75-85%	100-110%	81-91 h

Recently, members of the WHO network of collaborating laboratories on SARS have published the results of the first studies on the resistance of the SARS CoV against environmental factors and disinfectants (Annex 2). Although very preliminary, initial conclusions from these studies are that the virus is stable in faeces (and urine) at room temperature for at least 1-2 days. The virus was more stable (up to 4 days) in stool from a diarrhoea patient (which had higher pH than the normal stool samples tested).

Only a minimal reduction in virus concentration after 21 days at 4°C and -80°C was observed confirming the high resistance of the SARS CoV to low temperatures as seen for other coronaviruses. The reduction in virus concentration at a stable and constant room temperature for 2 days was one log only. This would indicate that the virus is more stable than the known human coronaviruses under these conditions. Heat at 56°C kills the SARS CoV at around 10 000 units per 15 min which is considered a rapid reduction.

It was also found that the virus loses infectivity after exposure to different disinfectants and fixatives commonly used in laboratories (Table 4.4). Chemical disinfectants used in the food industry are used at much lower concentrations and some of the compounds used in laboratories are not permitted in contact with foods. Thus new studies are needed on compounds commonly used in the food industry, water processes and at concentrations used under these conditions.

4 RESEARCH NEEDS

4.1 Detection

4.1.1 Introduction

Recent reports have shown the presence of SARS CoV in stools of a high proportion of people with SARS. Diarrhoea has been reported as a symptom in different proportions of cases in different regions. Research is needed to identify / detect the kinetics and level of faecal shedding during the course of infection with SARS, including levels of shedding, duration of shedding, pre- and asymptomatic transmission. Research should also assess the causal role of SARS CoV infection in diarrhoea, by ruling out common causes of enteric illness (particularly common in crowded healthcare settings). Contact monitoring (quarantine) should be done for diarrhoeal diseases.

To understand what happens to the virus outside the host, such as when it is shed in faeces, detection methods are critical. The lack of methodology in relation to detection of viruses in general has probably resulted in under-reporting of viral disease transmitted by sewage, food or water. Detection methods that distinguish between live and dead virus are needed to show that a virus can persist for a minimum period of time to be a health threat. It is impossible to anticipate all possible modes of viral transmission. However, when a new mode or vehicle of transmission is implicated the availability of appropriate methods of both viral detection and quantification can greatly contribute to facilitate the increase in the knowledge base.

4.1.2 Available methods

There are currently two methods available for detection of the SARS CoV in clinical samples/specimens.

1) Tissue culture method (involves infection/growth)
2) Molecular method based on PCR (only detection – no growth involved)

These methods are still sub-optimal and differ in their sensitivity with the molecular method currently more sensitive than tissue culture. Validated diagnostic tests in which we can be confident are critical for future work in this area. In addition, tests that can be used to detect and quantify the virus in samples such as sewage and water need to be more sensitive than those for clinical samples. Such methodology can be used to support and/or confirm epidemiological data, undertake studies on persistence and inactivation of the virus and validate management systems for the control of the virus.

4.1.3 Development of detection methods

Standardization of methodology would allow comparability of work undertaken in different laboratories. The current molecular method detects the virus but it does not provide information on the infectivity of the virus. Better cultural methods are needed to determine infectivity. In addition, in vivo methods are needed to provide information on dose-response. Methods for quantification of the virus are also important, for example in studies on persistence and inactivation of the virus.

4.1.4 Biosafety requirements

Working with SARS CoV requires a high level of biosafety. This along with the sophisticated nature of the detection methodology means that only specialized laboratories can currently undertake work on this virus. WHO has provided some guidance with regard to the level of biosafety required for working with SARS CoV (WHO biosafety guidelines for handling of SARS specimens: 25 April 2003; www.who.int). However, it was felt that this is not currently specific enough and open to different interpretations. The meeting recommended that the guidelines be reviewed and clarified if necessary. This is a new area of work and global standardization of working procedures from an early stage would facilitate progress and help ensure that all work proceeds in a harmonized manner. Some of the issues to be considered in relation to biosafety include the type of work being undertaken on the virus – for example is the objective of the work only to detect the virus or is there potential for an increase in viral load or a change in virulence. Studies on animals, for example, will require additional measures to be taken in relation to containment.

4.1.5 Other issues to be considered when developing detection methods

1) *Use of surrogates*

Because of the high level of biosafety required when working with SARS CoV, undertaking work on the development of detection methods can be done to a certain extent through the use of surrogates. This is more fully considered in section 4.2.

2) Detection of virus in different specimens
Isolation of virus from a specimen can be influenced by the composition of the sample and the presence of other micro flora. Therefore, in developing detection methodology consideration needs to be given to the type of sample that is being tested and how the virus can be extracted from faeces, water, food or aerosol droplets.

3) Consideration of existing methodology
Numerous methods for the detection of different viruses have been developed (e.g. HMSO: Methods for the isolation and identification of human enteric viruses from waters and associated materials 1995: Methods for the examination of Waters and Associated Materials). The utility of these methods (e.g. absorption onto filters) should be considered / evaluated for the detection of SARS CoV. For food, there are few reliable and validated detection methods.

4) Quantification
Methods that allow quantification of the virus are needed to allow enumeration of the virus for example in the environment, in studies on the effect of environmental conditions on the environment and dose.

4.2 Use of surrogates

The use of valid surrogates for SARS CoV provides a lower cost means of developing methodology, as well as possibly undertaking some behavioural studies, by reducing the level of biosafety required. This allows more laboratories to work in the area and potentially speed up the development of new methods. However, methodology developed using surrogates will have to be validated using SARS CoV. Depending on the studies to be undertaken it might be appropriate to use different surrogates. For some studies, an index virus (a virus with similar reaction to a particular process) could be used. In other areas a surrogate virus (a virus that would mimic the behaviour of the SARS CoV would be needed.

As initial studies seem to be indicating that the SARS CoV is quite different from other coronaviruses, it is very difficult to anticipate which virus can be used as a surrogate. Some of the issues to be considered when selecting surrogates include:

- Lower level of biosafety required to work with virus
- Similarity to the SARS CoV in terms of causing respiratory and /or enteric infection
- Use of comparative studies between SARS CoV and some possible surrogates to determine which would be the most appropriate.
- Inactivation by different agents (UV, heat, irradiation, etc.). Methods of inactivation are likely to affect SARS CoV in different ways. A profile of the inactivation pattern of potential surrogates compared to SARS CoV could be quite useful. This could also be used as a means of validating the selection of particular surrogates.
- Surrogate agents should be available at high viral titres. These are needed for studies on inactivation or persistence (may need to be able to see up to a 4 log reduction). Therefore, it is important that surrogates can be propagated in cell culture and can be detected.

Surrogates are also needed to undertake studies on the effect of the virus on the host. Such studies cannot be undertaken in humans; therefore it is necessary to develop an appropriate animal model.

Such a model could be used in studies to determine the infectious dose. Due to the similarity of the GI tract of pigs to that of humans, these animals may provide a suitable in vivo model (when fed with human food). But like the viral surrogates the use of this type of model would also have to be validated.

4.3 Persistence of SARS CoV in the environment

4.3.1 Introduction

The primary route of transmission and infection of SARS CoV has to date been observed to be respiratory. However, SARS CoV has been detected in faeces and was seen to survive up to 4 days in diarrhoea[2] and some initial studies on its survival in different stool suspensions have been undertaken (Annex 1). In one particular location, Amoy Gardens in Hong Kong, sewage has been hypothesized as the vehicle of transmission. This potential for SARS CoV to be introduced into the environment warrants consideration of the effect of environmental conditions on SARS CoV, its potential to persist in the environment and be transmitted by routes other than those currently recognized (refer to earlier text on current status). Initial studies on SARS CoV indicate that this virus is more stable than other human coronaviruses (Tables 3.1 – 3.4). However, most of the work to date has focussed on survival of the virus in clinical samples and the conditions they are exposed to rather than survival in the environment.

Table 4.1: Stability of SARS CoV in a controlled laboratory setting

a) Virus titre after different times of incubation at room temperature

Time	Titre of dried virus preparation	Titre of virus in solution
0 hours	10^3 / 100 ul	10^3 / 100 ul
3 hours	10^3 / 100 ul	10^2 / 100 ul
6 hours	10^2 / 100 ul	10^3 / 100 ul
24 hours	10^2 / 100 ul	10^3 / 100 ul

b) Virus titre after different times of incubation at room temperature with humidity of about 75%

Time	Titre of dried virus preparation	Titre of virus in solution
0 hours	10^3 / 100 ul	10^3 / 100 ul
3 hours	10^3 / 100 ul	10^2 / 100 ul
6 hours	10^3 / 100 ul	10^3 / 100 ul
24 hours	10^3 / 100 ul	10^3 / 100 ul
48 hours	10^2 / 100 ul	10^3 / 100 ul

*Starting virus titre 10^3 / 100 ul

*The virus preparation used was virus in tissue culture medium with 1% foetal calf serum (as a source of protein).

*Conclusion: The virus seems quite stable at room temperature for 2 days, and in this regard, it is more stable that the known human coronaviruses.

*Source: J.S.M. Peiris and K.H. Chan, Department of Microbiology, HKU & QMH, 13 April 2003

[2] Survival data should be expressed in log reductions

Table 4.2: Effect of heat on virus viability.

Temperature	Loss of virus infectivity in solution after heating at different times and temperature			
	15 minutes	30 minutes	45 minutes	60 minutes
30°C	0	0	0	0
37°C	0	0	0	0
56 ^{o}C	$\geq 10^4$	$\geq 10^4$	$\geq 10^4$	$\geq 10^4$
70 ^{o}C	$\geq 10^4$	$\geq 10^4$	$\geq 10^4$	$\geq 10^4$

*Starting virus titre 10^4 / 100 ul

*The virus preparation used was virus in tissue culture medium with 1% or 2% foetal calf serum (as a source of protein) depending on the temperatures tested.

*Conclusions: Heat at 56°C kills at least 10,000 infectious virus doses in 15 minutes but temperatures of 37 ^{o}C or lower do not kill virus effectively.

*Source: J.S.M. Peiris and K.H. Chan, Department of Microbiology, HKU & QMH, 13 April 2003

Table 4.3: Effect of stool/urine on virus viability.

Sample	Loss of virus infectivity in solution after heating at different times of incubation at room temperature					
	0 hour	1 hour	3 hours	6 hours	24 hours	48 hours*
Stool	10^3	10^3	10^3	10^3	10^{3*}	10^{2*}
Urine	10^3	10^3	10^3	10^3	10^3	nd

*Starting virus titre 10^3/ 100 ul

* Incubated at 4^0 C to prevent outgrow of bacteria present in stool

*Conclusion: The virus is quite stable in urine or stool samples at room temperature for at least 1~2 days.

*Source: J.S.M. Peiris and K.H. Chan, Department of Microbiology, HKU & QMH, 13 April 2003

Table 4.4: Effect of different fixatives or chemical disinfectants on virus viability.

	Loss of virus infectivity in solution of different fixatives or disinfectants after 5 minutes of incubation at room temperature						
Fixatives/ Disinfectants	Acetone	10% Formaldehyde	10% Paraformaldehyde	AVL	10% Clorox	75% ethanol	2% printol
5 minutes	$\geq 10^3$	$\geq 10^3$	$\geq 10^3$	$\geq 10^3$	$\geq 10^3$	$\geq 10^3$	$\geq 10^3$

*Starting virus titre 10^5/ 10 ul

*Conclusion: The virus losses infectivity after exposure 5 minutes with different fixatives or disinfectants

*Source: J.S.M. Peiris and K.H. Chan, Department of Microbiology, HKU & QMH, 13 April 2003

4.3.2 Information needs

To better understand the possibility of the virus being transmitted via other routes there is a need to know more about the introduction into (i.e. excretion patterns -level of shedding, ratio, duration, post symptomatic) and the ability of the virus to persist in the environment. For example if SARS CoV were to be waterborne a certain level of persistence in the environment would be needed.

Although experiments are ongoing (mice in France, monkeys in the United States) there is no evidence of oral transmission of SARS CoV to date. Spread via fomites also seems to be an issue but there is no evidence to date that this is via an oral route.

Some initial work has been carried out in the area of viral survival and persistence (Annex 2) but there are a number of key questions that need to be addressed to determine if this virus can persist in the environment (sewage, food, water etc.) include:

1. Effect of pH
    - Coronaviruses can be resistant to pH of between 2 and 12 so persistence at a wide range of pH values needs to be considered

2. Effect of temperature
    - Temperature range to be studied will depend on whether looking at environmental persistence or disinfection
    - Persistence at a wide range of temperatures needs to be considered for environmental studies
    - If studies were to be undertaken for food, temperatures used for food processing would need to be considered
    - The viruses do persist at low temperatures
    - The matrix in which the virus is present may influence its sensitivity to temperature

3. Effect of salinity
    - Persistence in fresh water
    - Persistence in the marine environment

4. Presence in faeces
    - Some preliminary data available and work ongoing (Annex 1 & 2).
    - Current results are difficult to interpret
    - Some of the issues to be resolved include dealing with resident micro flora in the stool. More information is needed in this area

5. Persistence on fomites
    - Desiccation of viruses on different surfaces
    - Consideration may need to be given to the types of surfaces used in water transport, food preparation, processing

6. Effect of disinfection
    - Some consideration has been given to the effect of disinfectants but mainly to those used in hospital, laboratory settings
    - Issues to be considered include disinfectants used in water and food areas and the lower concentrations at which they are used

7. Effect of light
    - Consideration should be given in particular to UV light

8. Effect of sediment
    - Consider presence of particulate matter as this usually enhances survival of viruses

9. Movement from sewage to persons, water, or food etc
    - Information is needed on the concentration of virus that moves from one vehicle to another – i.e. quantification of virus

Addressing these questions should contribute to our understanding of how the virus survives outside of the host, in the absence of host cells and what factors facilitate this survival.

4.3.3 Inactivation (potential to persist in controlled environments e.g. water treatment or food processing plants)

Consideration needs to be given as to whether SARS CoV is any more resistant to inactivation than other coronaviruses. Work needs to be undertaken on the effect disinfectants such as those used in food processing environments. These include quaternary ammonia compounds and phenolics, which are very effective against bacteria but useless against non-enveloped viruses. However, little has been done on their effect on enveloped viruses but it is thought that if the right disinfectant/detergent is used SARS will be inactivated. There are some phenolics that have been registered as being active against coronaviruses. Some of this work undertaken to support such registrations should be revisited at this time as SARS CoV is not behaving in the same way as human coronaviruses but may be more similar to animal coronaviruses. In undertaking inactivation studies consideration should be given to the types of compounds used in the food industry and the concentrations in which they are used.

In relation to the food-processing environment this could be examined from relevant angles. A list of commonly used processes in food industry and their effect on viruses has been compiled (Table 3.3). Such a table could be useful in determining risk factors in relation to SARS. For example, information or current knowledge in relation to the most resistant viruses could be used as a starting point for providing guidance i.e. if a process kills the most resistant viruses then it would also be effective against SARS CoV. Food processes that result in virus inactivation could be considered as potential critical control points in a HACCP plan. Apart from the food processing environments other issues that may need to be considered include the effect of food properties and potential inactivators such as enzymes, for example the effect of proteases in meat on the virus. Other viruses in meat however seem to escape proteases. If foods were to be examined they should first be categorized, as not all foods provide the same opportunity for contamination by and persistence of the virus.

As fomites seem to play a role in virus transmission another area for consideration may be the potential of food processing equipment to harbour the virus. This would require studies of a series of different materials used to build food-processing equipment to see how virus survives on different surfaces

5 SCENARIOS

The scenarios described in this section are developed in an attempt to guide research based on several potential future developments in our knowledge.

5.1 SARS CoV detected in sewage / stools

5.1.1 Current Situation

- Established: SARS CoV recovered from stools -
- Persistence in faeces for up to 4 days, Detected in toilets for up to7 days
- Droplet (or aerosol) transmission of SARS CoV through sewage (hypothesis based on Amoy Gardens epidemiological situation)

5.1.2 What we need to know

- Infectious status of virus in stools
- Viral load – how much is being excreted (10^6? – more investigation needed) (type of infection – enteric or clearance of respiratory infection)
- Quantitative information re persistence of virus
- % people exhibiting diarrhoeal symptoms (Amoy Gardens – 70%)
- excretion by asymptomatic cases, shedding by recovered patients
- Infectious dose or dose-response relationship
- Is enteric infection possible – Intra-gastric inoculation of monkeys
- Association of SARS CoV with enteric illness (providing greater opportunity for spread)

5.1.3 Potential impact

- Faecal shedding can by some route lead to transmission of SARS (depends on outcome of Amoy garden investigation)
- Occupational hazard
- Opens door to consideration of other scenarios

5.1.4 Will current control measures suffice

The scope of concerns related to occurrence of virus in stools includes:

- The majority of the world's population uses (e.g. latrines). Such sanitation options may provide containment (protective effect) of virus-containing faeces. If faecal-oral transmission is demonstrated then hygiene related transmission will be of interest, and exacerbated if water for hygiene is not available.
- Buildings served with waterborne sewage provide an effective means of isolating household members from faecal material. If faecal-oral transmission is demonstrated then personal hygiene will be important in control. However, Amoy Gardens suggests that in some circumstances inhalation exposure may lead to infection and adequacy of building plumbing may therefore be important in control. Therefore, virus transmitted to centralized sewage may become a downstream hazard, depending on persistence.
- Where centralized sewage is the means of sanitation, if the faecal-oral or faecal-inhalation is confirmed then occupational exposure to sewage, sewage treatment and allied workers through both ingestion and droplet inhalation may require review.
- Many SARS patients develop [severe] diarrhoea. (Table: Summary of occurrence of diarrhoea among SARS patients from existing clinical descriptions from each country outbreak). Procedures for safe handling of faeces would need to be reconfirmed in light of developing understanding, of faecal-inhalation transmission.

5.1.4.1 Recommendations

- Further research on aerosol transmission associated with sewage (Assess risk associated with an Amoy Garden like situation – sewage handling (droplet transmission))
- Reconsider case definition for SARS case finding and contact tracing in light of indication of transmission by virus-containing faecal material, either directly or via environmental contamination (e.g. could cases occur without direct contact with infected persons).
- Reconsideration of guidelines for food workers after illness, depending on shedding of virus post-recovery

5.2 Possibility of faecal-oral transmission established

5.2.1 Current Situation

- Not established

5.2.2 What we would need to know if established

- Stability of the virus
- Survival/persistence under different environmental conditions, along the food & water chains
- Inactivation rates
- Quantitative information on viral persistence, inactivation for use in risk assessment
- Establish potential vehicles of transmission
- How widespread is faecal mode of transmission
- Key features of the disease
- Information on dose-response (ingestion, inhalation)
- Whether this mode of transmission existed since the initial emergence of SARS or if it is a new route of transmission as a result of some change in the virus.
- Detection techniques, their validation

5.2.3 Potential impact

- Consideration of other scenarios - opportunity for food / waterborne transmission
- Refocus on populations of concern
- Consideration of seasonal impact, possible reservoirs
- Need to properly assess importance and potential for prevention of transmission through hygiene behaviours

5.2.4 Will current hygiene practices suffice

- Human excreta and sewage is used as a fertilizer and water source in food production in many countries. There is little control of this practice, and therefore potential for contamination

5.2.5 Recommendations

- Strengthen surveillance systems directed at early detection of food- or water related illness
- Provide guidance on research needs (key features of the disease, environmental persistence)
- Look at chain in relation to food and water and identify potential hot spots -
- Use of modelling, risk assessment approach to assess and try to quantify potential risk
- Identification of data gaps, critical areas of intervention

5.3 Possibility of waterborne transmission established

5.3.1 Current Situation

- Not observed

5.3.2 What do we need to know if established

- Considering 2 scenarios – aerosols (inhalation) and ingestion
- Aerosol formation

- Consider difference in exposure scenarios – is water piped, treated, management systems in place
- Validate water controls using surrogates across full supply chain.
- Establish presence of SARS in water supplies – potential risk (risk assessment)
- Consider different water types and uses – i.e. consideration of recreational water (coastal, freshwater, swimming pools); irrigation; aquaculture; ingestion and aerosol generation.
- Dose-response in relation to ingestion and inhalation exposure

5.3.3 Potential impact

- Potential for very large outbreaks
- Impact on shellfish industry, aquaculture
- Potential high significance in poor households where water handling occurs.
- Loss of confidence in water supplies in cities.

5.3.4 Will current practices suffice

- Additional controls may not be needed but validation of controls already employed is important (both ingestion and inhalation exposures)

5.3.5 Recommendations

- Coordinate and promote development of appropriate sample handling (concentration, extraction) for water, sewage and related matrices
- Establishment and dissemination of guidelines
- Strengthen surveillance systems directed at early detection of food- or water related illness

5.4 Possibility of foodborne transmission established

5.4.1 Current Situation

- Not observed

5.4.2 What do we need to know if established

- As mentioned previously
- Some key features of the disease – are people still shedding when healthy? Pre- post- and asymptomatic shedding of the virus (faecal and respiratory)

5.4.3 Potential impact

- Increased risk to consumers
- Adverse effect on food trade
- Occupational risk to food workers (aerosol transmission)

5.4.4 Will current food hygiene practices suffice

- Additional controls may not be needed but validate what is currently in place
- Need to consider whether current measures sufficient to address possible food contamination via aerosols (respiratory infection)
- Review of CCPs with a view to viral contamination

5.4.5 Recommendations

- Strengthen surveillance systems directed at early detection of food- or water related illness
- Efficient interaction between risk assessors and risk managers on a real-time basis
- Categorization of foods according to risk
- Establishment / dissemination of guidelines – leadership role

6 FUTURE RESEARCH COORDINATION STRATEGY

Networks provide an important means for rapid sharing of information. A network of people with expertise in the environmental area in relation to viruses can provide a means of bringing information and knowledge together in a rapid manner to facilitate advancement in this area. A lot of information on SARS CoV is not yet available but as a large number of studies are ongoing more information will come on line in the near future and it would be useful to have a formulated coordinated structure to consider such information and provide further suggestions and recommendations. Such a group / network could provide factual information regarding information currently circulating on survival and inactivation, and environmental resistance. That information could then provide a basis for more targeted and coordinated studies.

A password protected electronic network could be coordinated by WHO. It would be useful to link this with existing electronic networks on SARS epidemiology and laboratories. WHO or FAO are not funding agencies, but provide a scientific basis for the research to be undertaken. Funding is required for experimental / research work.

6.1 Prioritization of research needs

1. Stool shedding – role of sewage in context
2. Proof of faecal – oral transmission
3. Evidence of waterborne transmission
4. Evidence of foodborne transmission

7 RECOMMENDATIONS FOR COMMUNICATION AROUND THE RISK ISSUE OF SARS COV IN FOOD AND WATER.

7.1 Basic Principles

In general the move from satisfaction with “absence of evidence” to the requirement for “evidence of absence” of significant risk is guided by:

1) Strong public concerns about the potential of a significant risk.
2) Any “prima facie” scientific evidence that indicates the potential of a significant risk, and suggests how further evidence could be sought.

If either or both of these conditions are met, the justification for engaging in research is the need to prepare a “scientific basis” for the evaluation of whether a significant risk is likely. A conclusion of “no significant risk” is most credible when based on a firm body of scientific research.

The key factor related to SARS CoV in this area is: The need to approach the SARS problem based on science requires the early move to a mode of engagement that includes a scientific research agenda. At the same time a weighing of the need to substantiate the default assumption that there is not a significant risk against a suspicion of a potential significant risk needs to be clearly expressed.

An approach along these lines is precautionary in the sense that if it turned out that the default presumption was in error, this approach would be proactive in identifying this error. It is the most responsible way to approach such issues based at the same time on science and a precautionary approach.

It is important that any strategy for communication attempts to frame the specific issue within the bigger picture of the SARS outbreak, and explains what is being done in addition to what is known. The window of opportunity to prevent SARS from becoming endemic re-enforces the need to address the environmental issues in a structured manner.

7.2 Specific Approach

Pursuing a research agenda in this area, even without specific epidemiological evidence of transmission routes other than close contact, represents a proactive rather than a reactive research based approach. A specific approach can be spelled out in the context of each of the scenarios identified in this report (see sections 5.1 to 5.4):

A. It is clear that conditions (1) and (2) above have both been met in relation to the question of engaging in a coordinated research effort on SARS in food and water.

- The containment and management of SARS is now a major public concern.
- There are scientific data that present at least a prima facie case for potential risk in food and water.
- Preliminary studies show persistence of the SARS CoV in faeces and sewage. This establishes the possibility of transmission of the virus to food and water. It does not, however, suggest a new mode of transmission of the disease (in addition to close contact).
- Preliminary outcome of the Hong Kong Amoy Gardens investigation raises the possibility of transmission to humans via sewage. It does not, however, suggest the specific infectious route in this case.

B. The Amoy Gardens incident itself establishes the need for further research on the issue of potential transmission of SARS through sewage and faeces. It also suggests a potential evaluation of risk management measures needed (evaluation already ongoing).

C. The evidence of persistence of SARS virus in faeces justifies further research on the faecal-oral transmission issue (research already ongoing).

D. If a faecal-oral transmission route is suggested from credible research data, SARS would likely become a food safety issue. Such potential research data is, however, not likely to answer the question of whether a significant risk exists.

E. If a faecal-oral transmission route is suggested from credible research data, SARS would likely become a water safety issue. Such potential research data is, however, not likely to answer the question of whether a significant risk exists.

The underlying assumption of the approach here is the need to formulate messages that are:

- responsive to public concerns and questions
- truthful
- scientifically accurate – including the fair representation of the levels of uncertainty in the scientific evidence at any given time.
- based on the clear rationale of the need to substantiate with good science the presumption of "no significant risk" (not zero risk)
- not based on the unsubstantiated fear of significant risk.

ANNEX 1: SURVIVAL TIME OF SARS COV (ISOLATE 6109) IN DIFFERENT STOOL SUSPENSION

Source: Government Virus Unit, Department of Health, Hong Kong

Method used:

1. Choose four different kinds of norovurs negative stool
2. Prepare 10% or 20% stool suspension in PBS
3. Centrifuge at 1500g 20 min
4. Add 0.2 ml coronavirus to 1.8 ml stool suspension
5. Add 0.2 ml coronavirus to 1.8 ml transport medium as a control
6. After standing at room temperature for 0.5 hour, 1.5 hours, 3 hours, 6 hours, make serial 10-fold dilution of different stool suspension and transport medium in Earl's diluent.
7. Add 50ul of each virus dilution in different stool suspension or transport medium to four wells of a 96-well plate
8. Add 50ul 5%MM with trypsin to each well
9. Add 100ul 5%MM with trypsin to the cell control wells
10. Add 100ul Vero E_6 cells in 5%MM with trypsin to all the wells
11. Seal the plates
12. Incubate at 37ºC in CO_2 incubator
13. Observe daily
14. Repeat the 10-fold serial dilution of different stool suspension and transport medium on the consecutive days to obtain the end-point

Results:

	10% stool (lab no 5125) +CV	20% stool (lab no 5889) +CV	10% stool (lab no 5889) +CV	10% stool (lab no 5190) +CV	10% stool (lab no 5754 +CV	Transport medium +CV
0.5 hour	10^{3}	$10^{2.25}$	10^{3}	$10^{3.75}$	$10^{3.75}$	$10^{4.75}$
1 hour	$10^{1.5}$	$10^{1.5}$	$10^{1.5}$	10^{3}	$10^{3.25}$	10^{4}
3 hours	$<10^{0.5}$	$<10^{0.5}$	$10^{0.75}$	$10^{2.75}$	$10^{3.75}$	$10^{4.5}$
6 hours	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	10^{2}	$10^{3.5}$	$10^{4.75}$
1 day	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$10^{2.75}$	10^{4}
2 days	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$10^{2.5}$	$10^{2.75}$
4 days	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$10^{0.75}$	$10^{1.5}$
5 days	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	10^{2}
6 days	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$10^{1.5}$
7 days	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	$<10^{0.5}$	10^{1}
pH	6-7	7-8		8	9	8
Remarks	Stool from a six month old baby				Watery stool	

Survival time of Sabin poliovirus in different stool suspension

Source: Government Virus Unit, Department of Health, Hong Kong

Method used:

1. Choose four different kinds of norovirus negative stool
2. Prepare 10% or 20% stool suspension in PBS
3. Centrifuge at 1500g 20 min
4. Add 0.2 ml poliovirus to 1.8 ml stool suspension
5. Add 0.2 ml poliovirus to 1.8 ml transport medium as a control
6. After standing at room temperature for 0 hour, 3.5 hours, 6 hours, make serial 10-fold dilution of different stool suspension and transport medium in Earl's diluent.
7. Add 50ul of each virus dilution in different stool suspension or transport medium to four wells of a 96-well plate
8. Add 50ul 5%MM with trypsin to each well
9. Add 100ul 5%MM with trypsin to the cell control wells
10. Add 100ul Vero E_6 cells in 5%MM with trypsin to all the wells
11. Seal the plates
12. Incubate at 37ºC in CO_2 incubator
13. Observe daily
14. Repeat the 10-fold serial dilution of different stool suspension and transport medium on the consecutive days to obtain the end point

Results

	10% stool (lab no 5125) +polio	20% stool (lab no 5889) +polio	10% stool (lab no 5889) +polio	10% stool (lab no 5190) +polio	10% stool (lab no 5754 +polio	Transport medium +polio
0 hour	$10^{5.25}$	$10^{5.5}$	$10^{5.75}$	$10^{5.5}$	10^{5}	$10^{5.5}$
3.5 hours	$10^{3.5}$	10^{4}	$10^{4.75}$	$10^{4.25}$	$10^{4.25}$	$10^{4.5}$
6 hours	10^{4}	$10^{4.25}$	$10^{4.25}$	$10^{4.25}$	$10^{4.25}$	$10^{4.25}$
1 day	$10^{2.25}$	$10^{4.25}$	$10^{4.5}$	$10^{4.5}$	$10^{4.5}$	$10^{4.5}$
3 days	10^{2}	$10^{4.5}$	$10^{4.5}$	10^{4}	10^{4}	$10^{4.5}$
4 days	$10^{1.25}$	$10^{4.5}$	$10^{4.5}$	$10^{4.5}$	$10^{3.75}$	$10^{4.75}$
5 days	$<10^{0.5}$	10^{4}	$10^{4.25}$	$10^{4.25}$	$10^{2.75}$	10^{5}
6 days	$<10^{0.5}$	$10^{4.5}$	10^{4}	10^{4}	$10^{2.5}$	$10^{3.5}$

ANNEX 2: FIRST DATA ON STABILITY AND RESISTANCE OF SARS COV COMPILED BY MEMBERS OF WHO LABORATORY NETWORK

http://www.who.int/csr/sars/survival_2003_05_04/en/index.html

The following table provides the first compilation of data on resistance of the SARS CoV against environmental factors and disinfectants. This information has been provided by Members of the WHO multi-centre collaborative network on SARS diagnosis[3]. More detailed information on methods utilized and material used is being compiled and will be available shortly. The major conclusions from these studies are:

Virus survival in stool and urine

- Virus is stable in faeces (and urine) at room temperature for at least 1-2 days.
- Virus is more stable (up to 4 days) in stool from diarrhoea patients (which has higher pH than normal stool).

Disinfectants and fixatives (for use in laboratories)

- Virus loses infectivity after exposure to different commonly used disinfectants and fixatives.

Virus survival in cell-culture supernatant

- Only minimal reduction in virus concentration after 21 days at 4°C and -80°C.
- Reduction in virus concentration by one log only at stable room temperature for 2 days. This would indicate that the virus is more stable than the known human coronaviruses under these conditions.
- Heat at 56°C kills the SARS CoV at around 10000 units per 15 min (quick reduction).

Lab*	Substrate	Initial viral count log_{10PFU}	Condition	Survival time	Method of testing viability
GVU	virus spiked in baby stool	1.00E+03	pH 6-7	3 hr	Virus isolation in cell culture
	virus spiked in normal stool	7.50E+03	pH 8	6hr	Virus isolation in cell culture
	virus in diarrhoeal stool	7.50E+03	pH 9	4days	Virus isolation in cell culture
QMH	stool	1.00E+03	Room Temperature	at least 2 days	Virus isolation in cell culture
	urine	1.00E+03	Room Temperature	at least 24 hr	Virus isolation in cell culture
	Virus culture medium+ 1% bovine serum	1.00E+03	on plastic surface in room temperature	at least 2 days	Virus isolation in cell culture
	Virus culture medium+ 1% bovine serum	1.00E+04	30-37 °C	at least 1hr	Virus isolation in cell culture
	Virus culture medium+ 1% fetal calf serum	1.00E+04	56°C	degradation of titre over time (10 000 infectious virus units in 15 min)	Virus isolation in cell culture
	virus in Acetone, 10% Formaldehyde and Paraformaldehyde, 10%	1.00E+06	Room Temperature	less than 5 min	Virus isolation in cell culture

[3] http://www.who.int/csr/sars/networkshome/en/

	Clorox, 75%ethanol, 2% phenol				
NIID	Virus culture+ 2% bovine serum	1.00E+06	minus 80°C	at least 4 days	Virus isolation and RT-PCR
	Virus culture+ 2% fetal calf serum	1.00E+06	4°C	at least 4 days	Virus isolation and RT-PCR
	Virus culture+ 2% fetal calf serum	1.00E+06	37°C	less than 4 days	Virus isolation and RT-PCR
	Virus culture+ 2% fetal calf serum	1.00E+05	56°C	less than 30min	
UniM	Virus culture	1.00E+06	4°C	at least 21 days	Virus isolation
	Virus culture	1.00E+06	minus 80°C	at least 21 days	Virus isolation

GVU: Government Virus Unit, Dept. of Health, Hong Kong, SAR China
QMH: Queen Mary Hospital, The University of Hong Kong, Hong Kong, SAR China
NIID: National Institute of infectious Diseases, Tokyo, Japan
UniM: University Marburg, Germany