NHRI INDUSTRY-ACADEMY COOPERATION

STUDY REPORT

Report code: 09D4-1IV12-1 *

Sponsor: BriseCare B.V. & Aether Services Taiwan LTD.

Study Laboratory: Chia-Chyi Liu's Lab, NHRI

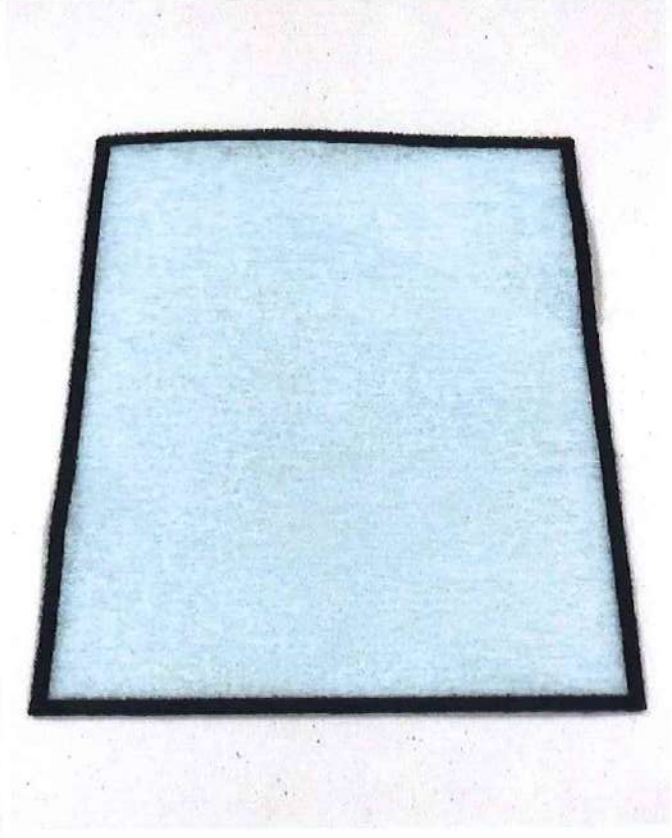
National Institute of Infectious Diseases and Vaccinology

National Health Research Institutes

Oct 30, 2020

STUDY REPORT

Date of Received	Sep/04/2020	Date of Study	Sep/07/2020 Oct/06/2020
Sponsor	BriseCare B.V. & Aether Services Taiwan LTD.	Client	Jerry Tsai

Name of Sample	Breathe Bio Filter	Number of Sample	1 package
Type and Specification	Polymer resin with chlorine dioxide coating	State of Sample	Blue filter
Batch Number	Mar/25/2020	Lot. Number	---
Sample Picture			
Items of Study	The virus stability of Enterovirus in artificial materials		
Remarks	Attachments: Protocol, Raw data, References		

STUDY REPORT (1/4)

Date of Received: Sep/04/2020

Date of Study: Sep/07/2020 to Oct/06/2020

Study Title: The virus stability of Enterovirus in artificial materials

1. Materials

1.1. Name of Sample: Breathe Bio Filter

1.2. Virus : Enterovirus A71 (EV-A71 E59 strain)

1.3. Dilution Buffer : Phosphate buffered saline (PBS)

1.4. Cell : RD cell

1.5. Culture Medium: DMEM + 10% FBS

2. Test Condition

2.1. Temperature : 24-26 °C

2.2. Humidity : 40-60 %

2.3. Time Point (Minute) : 10 min, 20 min, 30 min, 45 min, 60 min, 240 min, 480 min

2.4. Sample size : 5 x 20 mm rectangle

2.5. Reaction Environment: The sample and virus are reacted and mixed in a screw-cap plastic tube and placed in a biological safety cabinet

3. Result

Seeding Virus Titer: 31331 $TCID_{50}$/mL

Time Point (Minute)	Control Group (No filter) Virus Titer	Rate of Virus Degradation (%)	Test Group (With filter) Virus Titer	Rate of Virus Degradation with Sample (%)
10 min	51006.7	-62.79	ND	99.99
20 min	67666.7	-115.97	ND	99.99
30 min	38537	-22.99	ND	99.99
45 min	84806.3	-170.67	ND	99.99
60 min	58802.3	-87.68	ND	99.99
240 min	36160.3	-15.41	ND	99.99
480 min	47126	-50.41	ND	99.99

Remark: "ND" means not detect CPE.

Research Assistant: Yu-Sheng Shen

Principal Investigator: Chia Chyi Liu

Oct/3/2020

STUDY REPORT (2/4)

Date of Received: Sep/04/2020

Date of Study: Sep/07/2020 to Oct/06/2020

Study Title: The virus stability of Enterovirus in artificial materials

1. Materials

1.1. Name of Sample: Breathe Bio Filter

1.2. Virus : Coxsackievirus A6 (CV-A6 M0746 strain)

1.3. Dilution Buffer : Phosphate buffered saline (PBS)

1.4. Cell : RD cell

1.5. Culture Medium: DMEM + 10% FBS

2. Test Condition

2.1. Temperature : 24-26 °C

2.2. Humidity : 40-60 %

2.3. Time Point (Minute) : 10 min, 20 min, 30 min, 45 min, 60 min, 240 min, 480 min

2.4. Sample size : 5 x 20 mm rectangle

2.5. Reaction Environment: The sample and virus are reacted and mixed in a screw-cap plastic tube and placed in a biological safety cabinet

3. Result

Seeding Virus Titer: 9532.3 $TCID_{50}$/mL

Time Point (Minute)	Control Group (No filter) Virus Titer	Rate of Virus Degradation (%)	Test Group (With filter) Virus Titer	Rate of Virus Degradation with Sample (%)
10 min	9238.7	3.08	301.7	96.83
20 min	8637	9.39	187.3	98.02
30 min	8380.3	12.08	226.3	97.60
45 min	12207.3	-28.06	160	98.37
60 min	10083.7	-5.78	66.3	99.31
240 min	9777.3	-2.57	93.7	99.02
480 min	10120	-6.16	ND	99.99

Remark: "ND" means not detect CPE.

Research Assistant: Yu-Sheng Shen Principal Investigator: Chia Chyi Liu

Oct/30/2020

STUDY REPORT (3/4)

Date of Received: Sep/04/2020

Date of Study: Sep/07/2020 to Oct/06/2020

Study Title: The virus stability of Enterovirus in artificial materials

1. Materials

1.1. Name of Sample: Breathe Bio Filter

1.2. Virus : Coxsackievirus A10 (CV-A10 M2014 strain)

1.3. Dilution Buffer : Phosphate buffered saline (PBS)

1.4. Cell : RD cell

1.5. Culture Medium: DMEM + 10% FBS

2. Test Condition

2.1. Temperature : 24-26 °C

2.2. Humidity : 40-60 %

2.3. Time Point (Minute) : 10 min, 20 min, 30 min, 45 min, 60 min, 240 min, 480 min

2.4. Sample size : 5 x 20 mm rectangle

2.5. Reaction Environment: The sample and virus are reacted and mixed in a screw-cap plastic tube and placed in a biological safety cabinet

3. Result

Seeding Virus Titer: 137835 $TCID_{50}$/mL

Time Point (Minute)	Control Group (No filter) Virus Titer	Rate of Virus Degradation (%)	Test Group (With filter) Virus Titer	Rate of Virus Degradation with Sample (%)
10 min	147273.3	-6.85	ND	99.99
20 min	146717.7	-6.44	ND	99.99
30 min	147273.3	-6.85	ND	99.99
45 min	130087	5.62	ND	99.99
60 min	204371.3	-48.27	ND	99.99
240 min	160920	-16.75	ND	99.99
480 min	218573.7	-58.58	ND	99.99

Remark: "ND" means not detect CPE.

Research Assistant: Yu-Sheng Shen Principal Investigator: Chia Chyi Liu

Oct/30/2020

STUDY REPORT (4/4)

Date of Received: Sep/04/2020

Date of Study: Sep/07/2020 to Oct/06/2020

Study Title: The virus stability of Enterovirus in artificial materials

1. Materials

1.1. Name of Sample: Breathe Bio Filter

1.2. Virus : Coxsackievirus A16 (CV-A16 N5079 strain)

1.3. Dilution Buffer : Phosphate buffered saline (PBS)

1.4. Cell : RD cell

1.5. Culture Medium: DMEM + 10% FBS

2. Test Condition

2.1. Temperature : 24-26 °C

2.2. Humidity : 40-60 %

2.3. Time Point (Minute) : 10 min, 20 min, 30 min, 45 min, 60 min, 240 min, 480 min

2.4. Sample size : 5 x 20 mm rectangle

2.5. Reaction Environment: The sample and virus are reacted and mixed in a screw-cap plastic tube and placed in a biological safety cabinet

3. Result

Seeding Virus Titer: 15007.7 $TCID_{50}$/mL

Time Point (Minute)	Control Group (No filter) Virus Titer	Rate of Virus Degradation (%)	Test Group (With filter) Virus Titer	Rate of Virus Degradation with Sample (%)
10 min	14280.3	4.85	ND	99.99
20 min	14727	1.87	ND	99.99
30 min	14390.7	4.11	ND	99.99
45 min	14727	1.87	ND	99.99
60 min	14727	1.87	ND	99.99
240 min	22200	-47.92	ND	99.99
480 min	13643	9.09	ND	99.99

Remark: "ND" means not detect CPE.

Research Assistant: Yu-Sheng Shen Principal Investigator: Chia Chyi Liu

Oct/30/2020

Annex 1

The virus stability of Enterovirus in artificial materials

Study Protocol

1. Purpose

To study the stability of enterovirus that is adsorbed on artificial material.

2. Introduction

There are many viruses and bacteria in our life environment, some of which can cause diseases after infecting humans. Enterovirus is an important infectious disease in Taiwan. The epidemic cycle usually occurs in the summer. There have been several pandemics in the past 20 years, and that induce several severe and death cases of serious neurological diseases in young children. In order to prevent enterovirus infection, the government promotes the implementation of environmental cleanliness and key disinfection, and to eliminate the conditions in life that may facilitate the spread of enterovirus. In this study, a test method was established to investigate the enterovirus stability after the enterovirus was adsorbed on the artificial material. After the artificial material is cut to an appropriate size, the quantified virus solution is added, and samples are sampling at different times. Finally, the virus titer of enterovirus is determined to evaluate the degradation of the enterovirus on artificial material.

3. Scope of application

This method tests artificial product material samples. The materials can be plastic, paper, fiber and other materials. According to the sample type, an appropriate area test sample can be prepared and cut.

4. Test procedure

4.1. Test sample preparation

4.1.1. The sponsor provides the test artifact materials.

4.1.2. A rectangular block (1 cm^2) with an area of 5 x 20 mm is cut from the part of the product material as a test sample. When preparing the test sample should avoid contaminants, such as microbial contamination, mutual contamination between product materials, dirt, and biosafety.

4.1.3. The artifact materials are cut in a biosafety cabinet, and use sterilized metal scissors for cutting.

4.1.4. If the test sample is an autoclaved material. It is cleaned 3 times with ultrasonic cleaner (10 minutes each time), then sterilized in an autoclave, and dried in an oven. The test sample is putted into a 1.5 mL screw-cap plastic tube with sterilized

tweezers in the biosafety cabinet.

4.1.5. If the test sample is a non-autoclaved material. The test sample is putted into a 1.5 mL screw-cap plastic tube with sterilized tweezers in the biosafety cabinet.

4.2. Virus solution seeding

4.2.1. To avoid the risk of biohazard, virus preparation, virus is added into test sample, test placement and culture medium washing and sampling are all in a biosafety cabinet. The laboratory room is maintained in an environment of 24-26°C and 40-60% humidity.

4.2.2. The tested virus strains were thawed from the freezer at -80 °C, and diluted with phosphate buffered saline (PBS) to 1 x 10^4 - 1 x 10^5 TCID50/mL as virus solution before test.

4.2.3. Inoculate 1 mL of virus solution (1 x 10^4 - 1 x 10^5 TCID50) into a 1.5 mL screw-cap plastic tube containing the test sample, and three replicates for each test group.

4.2.4. The control group did not place the test sample. Inoculate 1 mL of virus solution (1 x 10^4 - 1 x 10^5 TCID50) into a 1.5 mL screw-cap plastic tube, and three replicates for each control group.

4.2.5. The screw-cap plastic tube is closed to prevent the virus spilling out.

4.2.6. The test within 1 hour or less, that plastic tube should be placed in a biosafety cabinet.

4.2.7. The test more than 1 hour, that plastic tube should be placed in a metal box to prevent UV light in a biosafety cabinet.

4.3. Sampling

4.3.1. According to the set sampling time point, that each screw-cap tube of the test group and the control group has been screwed tightly before being removed from the biological safety cabinet.

4.3.2. Sampling time points: 0 minutes, 10 minutes, 20 minutes, 30 minutes, 45 minutes, 60 minutes, 240 minutes, 480 minutes.

4.4. Sample storage

4.4.1. For the screw-cap tube containing the virus solution, the plastic tube should be confirmed that the sampling time point is marked.

4.4.2. Collect the screw-cap tube in -80 °C freezer for virus titer assay.

4.5. Virus titer determination

4.5.1. RD cells are seeded in a 96-well plastic plate (1 x10^4 cells/well).

4.5.2. After thawing the test tube, virus solution is diluted with DMEM (10-fold serial dilution from 10^{-1} to 10^{-8}).

4.5.3. Add 20 μL of the diluent to a well, and perform 6 replicates for each dilution.

4.5.4. Culture the 96-well plastic plate in the 37 °C, 5% CO_2 incubator.

4.5.5. Observe the cytopathic effect (CPE) on the 4th day, and stop on the 6th day.

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4.6. Data analysis and report

4.6.1. The number of wells that present CPE in each dilution of each sample are record into the Excel file.

4.6.2. Use the Reed-Muench method to calculate the virus titer of each sample.

4.6.3. Plot the virus titer at different time points between the test group and the control group.

4.6.4. Collect the data to complete the study report.

4.6.5. Record the type, size, shape and thickness of the test sample.

4.6.6. Record the types of cells and viruses, the virus titer of the virus solution, and the virus titer of the test group and the control group at each sampling time point.

4.6.7. Record the models of important instruments.

5. Material preparation:

5.1 Instruments:

5.1.1 Laminar flow cabinet

5.1.2. Biosafety cabinet (THERMO 1284 Class II A2: 102472-5172)

5.1.3. Cell culture incubator (THERMO 370 Steri-Cycle CO2 incubator: 304361-2236)

5.1.4. Micropipetman

5.1.5. Pipetman

5.1.6. Autoclave (HUXLEY HL-343: S0512019)

5.1.7. Water bath

5.1.8. Timer

5.1.9. Metal box

5.1.10. Metal scissors

5.1.11. Metal tweezers

5.2 Disposable materials:

5.2.1. 6-well plastic plate

5.2.2. 96-well plastic plate

5.2.3. Pipette

5.2.4. Micropipette

5.2.5. Centrifuge tube

5.2.6. Sterilization bag

5.2.7. Autoclave tape

5.2.8. Screw-cap tube

5.3. Cells and viruses:

5.3.1. Human rhabdomyosarcoma cells (RD cells)

5.3.2. Enterovirus A71 (EV-A71 E59 strain)

5.3.3. Coxsackievirus A group (CV-A6 M0746 strain; CV-A10 M2014 strain; CV-A16

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N5079 strain)

5.4. Medium and reagents:

5.4.1. DMEM

5.4.2. Antibiotics (for cell culture)

5.4.3. Fetal Bovine Serum (for cell culture)

5.4.4. Phosphate buffered saline (PBS)

6. Statistics:

6.1. The data of three replicates are averaged for each time point.

6.2. Calculate the rate of virus degradation and the rate of virus degradation with sample at each time point.

$$\text{The rate of virus degradation} = \frac{\text{Seeding virus titer} - \text{Virus titer of control group}}{\text{Seeding virus titer}} \times 100\%$$

$$\text{The rate of virus degradation with sample} = \frac{\text{Seeding virus titer} \times (1 - \text{The rate of virus degradation}) - \text{Virus titer of test group}}{\text{Seeding virus titer} \times (1 - \text{The rate of virus degradation})} \times 100\%$$

6.3. The calculated values are collected for the study report.

7. References：

7.1. RD 細胞適應培養(Adaptation)建立各型腸病毒標準病毒株庫 (DOH94-DC-2004).

7.2. Immunological and biochemical characterizations of coxsackievirus A6 and A10 viral particles. Antivir. Res. 2016; 129: 58-66.

7.3. 傳染病標準檢驗方法手冊. 衛生福利部疾病管制署. 2019 /Apr/16.

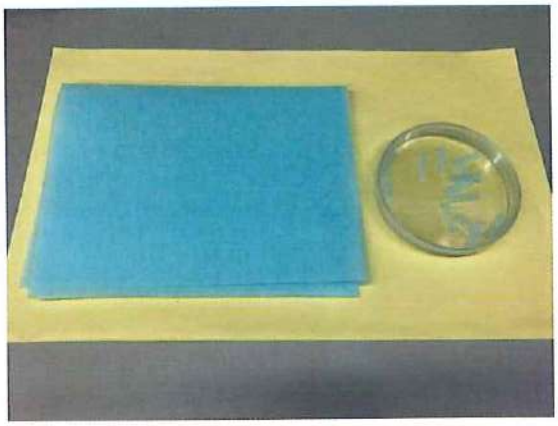
7.4. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 2020; 382(16):1564-1567.

7.5. CDC 腸病毒防治核心教材:
(https://www.cdc.gov.tw/Category/MPage/Vql2d6XqCySLOzXWaMdJqg)

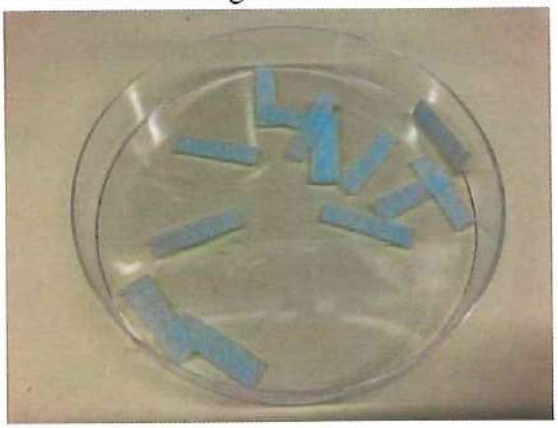
Annex 2

Study Title: The virus stability of Enterovirus in artificial materials
Material Treatment

1. Breathe Bio filter was cut with sterilized instruments.



2. Cut out a rectangular block with an area of 5 x 20 mm



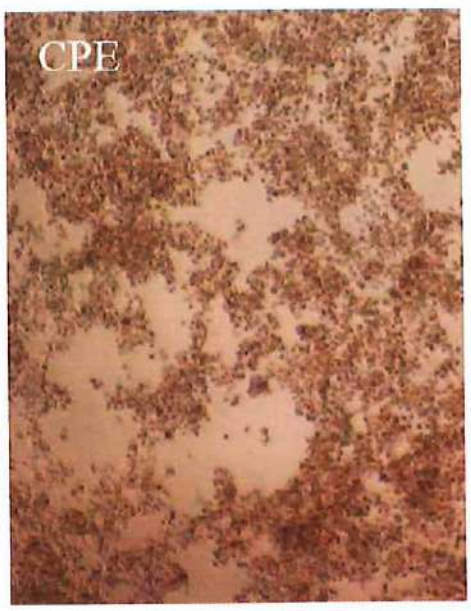
3. The cut out rectangular block can be completely infiltrated in 1mL virus solution. (Left tube is test group with filter, and right tube is the control group without filter).

Annex 3

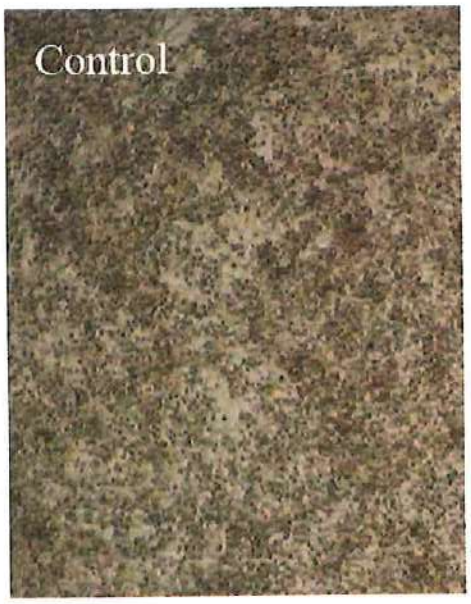
Study Title: The virus stability of Enterovirus in artificial materials
Atlas of Cytopathic Effect (CPE) with Enterovirus

After the test samples were added in RD cells, the cytopathic effect (CPE) was observed within 4-6 days.

EV-A71

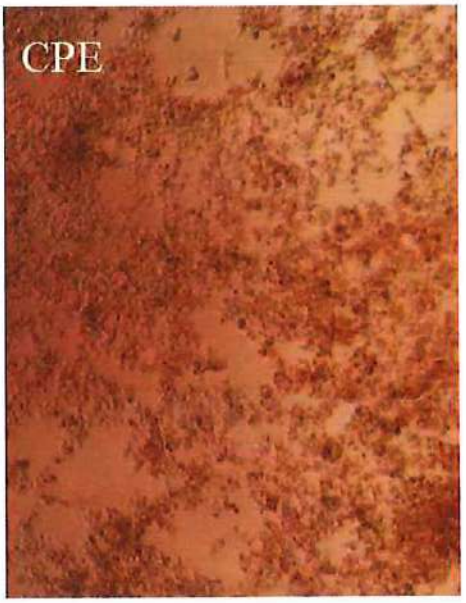


CV-A6

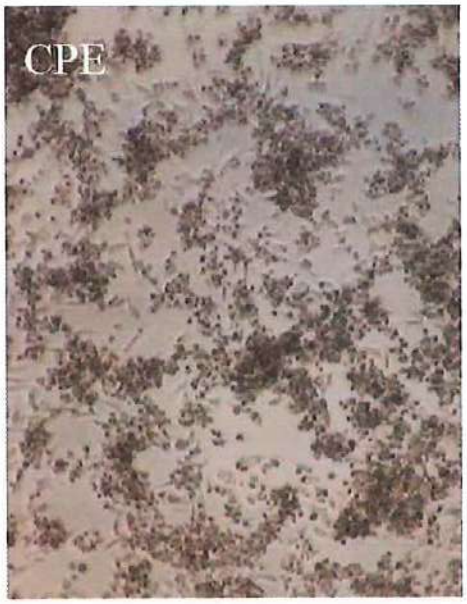


Annex 3

CV-A10



CV-A16



Annex 4

Study Title: The virus stability of Enterovirus in artificial materials

Virus: Enterovirus A71 (EV-A71 E59 strain)

Raw Data

Control Group (No filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	19005	71473	115506	28117	50000	37494	8891	71473
Sample 2	37494	50000	37494	37494	88913	50000	28117	19905
Sample 3	37494	31547	50000	50000	115506	88913	71473	50000
Average	31331	51006.7	67666.7	38537	84806.3	58802.3	36160.3	47126

Test Group (With filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	---	ND	ND	ND	ND	ND	ND	ND
Sample 2	---	ND	ND	ND	ND	ND	ND	ND
Sample 3	---	ND	ND	ND	ND	ND	ND	ND
Average	---	ND	ND	ND	ND	ND	ND	ND

Remark: "---" means not test ; "ND" means not detect CPE.

Rate of Virus Degradation (%) / Rate of Virus Degradation with Sample (%)

Time (minute)	0	10	20	30	45	60	240	480
Rate of Virus Degradation (%)	0	-62.80	-115.97	-22.99	-170.68	-87.68	-15.41	-50.41
Rate of Virus Degradation with Sample (%)	0	99.99	99.99	99.99	99.99	99.99	99.99	99.99

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Study Title: The virus stability of Enterovirus in artificial materials

Virus: Coxsackievirus A6 (CV-A6 M0746 strain)

Raw Data

Control Group (No filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	7147	5000	2811	3749	5000	2811	8891	1990
Sample 2	12559	19905	11550	11550	15811	12559	11550	15811
Sample 3	8891	2811	11550	9842	15811	14881	8891	12559
Average	9532.3	9238.7	8637	8380.3	12207.33	10083.7	9777.3	10120

Test Group (With filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	---	281	0	199	199	ND	ND	ND
Sample 2	---	374	281	199	ND	199	281	ND
Sample 3	---	250	281	281	281	ND	ND	ND
Average	---	301.7	187.3	226.3	160	66.3	93.7	ND

Remark: "---" means not test ; "ND" means not detect CPE.

Rate of Virus Degradation (%) / Rate of Virus Degradation with Sample (%)

Time (minute)	0	10	20	30	45	60	240	480
Rate of Virus Degradation (%)	0	3.08	9.39	12.09	-28.06	-5.78	-2.57	-6.16
Rate of Virus Degradation with Sample (%)	0	96.83	98.02	97.60	98.37	99.31	99.02	99.99

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Study Title: The virus stability of Enterovirus in artificial materials

Virus: Coxsackievirus A10 (CV-A10 M2014 strain)

Raw Data

Control Group (No filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	216438	158113	199053	158113	199053	216438	199053	216438
Sample 2	125594	125594	115506	125594	153735	115506	158113	281170
Sample 3	71473	158113	125594	158113	37473	281170	125594	158113
Average	137835	147273.3	146717.7	147273.3	130087	204371.3	160920	218573.7

Test Group (With filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	---	ND	ND	ND	ND	ND	ND	ND
Sample 2	---	ND	ND	ND	ND	ND	ND	ND
Sample 3	---	ND	ND	ND	ND	ND	ND	ND
Average	---	ND	ND	ND	ND	ND	ND	ND

Remark: "---" means not test ; "ND" means not detect CPE.

Rate of Virus Degradation (%) / Rate of Virus Degradation with Sample (%)

Time (minute)	0	10	20	30	45	60	240	480
Rate of Virus Degradation (%)	0	-6.85	-6.44	-6.85	5.62	-48.27	-16.75	-58.58
Rate of Virus Degradation with Sample (%)	0	99.99	99.99	99.99	99.99	99.99	99.99	99.99

Annex 4

Study Title: The virus stability of Enterovirus in artificial materials

Virus: Coxsackievirus A16 (CV-A16 N5079 strain)

Raw Data

Control Group (No filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	12559	8891	15811	15811	15811	15811	34978	12559
Sample 2	12559	8891	12559	15811	12559	12559	15811	12559
Sample 3	19905	25059	15811	11550	15811	15811	15811	15811
Average	15007.7	14280.3	14727	14390.67	14727	14727	22200	13643

Test Group (With filter): TCID50/mL

Time (minute)	0	10	20	30	45	60	240	480
Sample 1	---	ND	ND	ND	ND	ND	ND	ND
Sample 2	---	ND	ND	ND	ND	ND	ND	ND
Sample 3	---	ND	ND	ND	ND	ND	ND	ND
Average	---	ND	ND	ND	ND	ND	ND	ND

Remark: "---" means not test ; "ND" means not detect CPE.

Rate of Virus Degradation (%) / Rate of Virus Degradation with Sample (%)

Time (minute)	0	10	20	30	45	60	240	480
Rate of Virus Degradation (%)	0	4.85	1.875	4.115	1.875	1.875	-47.925	9.095
Rate of Virus Degradation with Sample (%)	0	99.99	99.99	99.99	99.99	99.99	99.99	99.99

Annex 5

Enterovirus / Enterovirus Infection with Severe Complications

Background

Enterovirus belongs to a group of small RNA viruses, including polioviruses, Coxsackie A viruses, Coxsackie B viruses, echoviruses, and other enteroviruses (EVD68~). EVA71 has a significantly higher pathogenicity compared to other known enteroviruses, especially regarding neurological complications. Enteroviruses are found in the gastrointestinal tract (the stool of infected persons, mouth) and respiratory tract (such as saliva, sputum, or nasal mucus). Infections can be caused by direct contact with the secretions of infected persons or with contaminated surfaces or objects. Neonatal infection may also be acquired vertically from an infected mother in utero or at the time of delivery. Humans appear to be the only known host and source for enteroviruses transmission. The patient is contagious before their onset, and the infectivity will last for weeks after the patient is recovered. There are currently no preventive vaccines for non-polio enteroviruses in Taiwan and no known highly efficacious medicine to eliminate the virus once it is inside the human body. Therefore, enteroviruses will continue to pose a threat to human health for the foreseeable future.

Epidemiology

According to survey data gathered over a period of several years by Taiwan CDC and the National Health Insurance (NHI) Administration, the number of weekly outpatient and emergency visits, as shown by the data transferred from the database of NHI, increases in late March and peaks around mid-June. It decreases after mid-June, and then decreases slowly. There is usually another smaller outbreak when schools reopen in September (see Figurev1).

Those survey data also indicate that children under the age of 5 are more prone to critical complications and death. In Taiwan, the case-fatality rate of enterovirus infection with severe complications (EVSC) are ranged from 1.3% to 33.3%. The major symptoms of enterovirus infection are herpangina and hand-foot-and-mouth disease (HFMD). EVA71 is the most commonly seen serotype of cases of EVSC in Taiwan.

EV/EVSC Surveillance in Taiwan?

1. Taiwan National Infectious Disease Statistics System–Enterovirus
2. Taiwan National Infectious Disease Statistics System–EVSC

Annex 5

3. School-Based Surveillance System

Prevention and Control

1. Established multiple and real-time surveillance systems for enterovirus infections, covering HFMD and herpangina, severe cases, clustering, virus isolation and typing.
2. Constructed a medical service network, including six regional chiefs, 77 responsible hospitals and eight contract laboratories.
3. Health Education and Inspection
    - (1)Local organizations work with the community to promote enterovirus education and prevention.
    - (2)Restaurants, schools, hospitals, clinics and other public gathering places must conduct regular inspections for environmental sanitation and provide hand-washing facilities.
4. Establishment of consultation channels staffed by clinical professionals. The professionals provide clinical health care consultation and guidelines for treating enterovirus complications. Primary care for patients with complications can effectively lower the mortality rate.
5. "The Manual for Enterovirus Prevention" and "The Handbook for Enterovirus Prevention for Child Care Workers" list all necessary precautions. These materials are provided on the Taiwan CDC website and updated timely.
6. Workshops are held on the clinical treatment of critical enterovirus complications to enhance doctors' skills in treating the disease, raise treatment quality and reduce mortality rates and sequelae.
7. To reduce the risk of EVSC clustering, the recommendation of class suspension has been revised for the pre-school education and care institutions, which are high-risk groups.
8. Strengthened implementation of infection control measures in hospitals and postpartum nursing care centers to reduce the risk of neonatal enterovirus clusters.

FAQs

1. **What are enteroviruses?**
    - Enterovirus is a general term of a group of small RNA viruses which accounts for more than 100 viruses, including

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Coxsackieviruses A, Coxsackieviruses B, polioviruses, echoviruses, and enteroviruses.

2. **Are these enteroviruses only found in Taiwan?What are the epidemic seasons?**
    - These kinds of viruses are usually cause epidemics in summer and early autumn all over the world. Because Taiwan is located in subtropics, the epidemics might take place throughout the year. According to historical statistics, May to June and September to October are the two major epidemic peaks in Taiwan.
3. **How do enteroviruses spread?**
    - Enteroviruses can be found on the respiratory secretions (e.g. saliva, sputum and nasal discharge) and stool of an infected person. People may get the infection by direct contact with secretions from an infected person or by indirect contact with contaminated surfaces or subjects. The virus may get into the body through respiratory tract or gastrointestinal tract. Neonatal infection may also be acquired vertically from an infected mother in utero or at the time of delivery.
    - Young children are often infected by direct or indirect contacts with adults who may have no symptom but still carry some viruses. They might also be infected by eating foods contaminated by virus-containing excrements. Toys are often the infecting intermediaries between young children, especially the hairy toys which cannot be cleaned routinely and have a higher risk of contaminated by potential pathogens.
    - Starting from several days before the appearance of symptom, patients who are infected by enteroviruses may transmit the virus to others and the peak of infectivity occurs within one week of disease onset. The virus can be found both in the throat and the stool. Patient may excrete the virus in the stools for several weeks. Enteroviruses transmitted easily among family members and the infection rate is especially high in crowded areas.
4. **What symptoms do enteroviruses cause?**
    - The incubation period of the enteroviruses infection is about 2-10 days. Most of the infected persons have no or very mild symptoms. Most patients will be fully recovered spontaneously with a few days.

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- Symptoms of characteristic enterovirus infection include vesicles and ulcers in the oral cavity, vesicles or papules on the palm or sole. Fever is frequently observed. The course of illness is usually 7-10 days. A small proportion of cases may be complicated by aseptic meningitis, encephalitis, myocarditis, pericarditis, pneumonitis and paralysis.

5. **Under what kind of situations one has to see a doctor immediately?**
   - If a child with enterovirus infection develops prodromal symptoms of complications such as flaccid paralysis, limb weakness, drowsiness, disturbed consciousness, inactivity, myoclonic jerk (repeated jerky-like movements similar to the startling response that involves abrupt muscle contractions of the whole body), continuous vomiting, tachypnea, and tachycardia, please ensure the child receive medical assistance at a large hospital as soon as possible.
6. **If a pregnant woman is infected with enterovirus, will she give birth of deformed babies?**
   - There is no evidence suggesting that enterovirus infection of the pregnant woman may lead to congenital anomaly of the baby. However, the potential risk cannot be totally excluded and pregnant women should avoid being infected as possible.
7. **Is there any special drug to treat enterovirus infections?**
   - Currently there is no drugs that can kill enteroviruses. Treatment strategies are mainly to support and stabilize the patient condition, and to relieve discomforts.
8. **Will one gain the immunity after being infected with the enterovirus?Will he be infected again later on?**
   - There are more than 100 types of viruses in the group of enteroviruses. One will get a long-lasting immunity once being infected with some enterovirus and the protection against that specific type of enterovirus may last for several decades. One disease entity may be caused by several types of enteroviruses. Therefore, some people may get hand-foot-and-mouth disease or herpangina more than once.
9. **How high is the mortality rate of enterovirus infections?**
   - Most patients with enterovirus infection have very mild or no symptoms. Although having not reached a general agreement, the fatality rate of enterovirus 71 infections has been estimated to be

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between 1/100,000 and 1/10,000 during the first outbreak of enterovirus 71 infection in Taiwan in 1998. In other words, after being infected with enterovirus 71, more than 99.9% of the patients will recover. However, it still caused quite a shock in Taiwan because the mortality rate of enterovirus 71 is higher than other common viral infections. Therefore, it is mandatory to have a thorough understanding of enteroviruses and the routes of their transmission to decrease the risk of infection.

10. **How to prevent enterovirus infections?**

- Currently there is no vaccine to prevent enterovirus infections except for polio viruses. Washing hands often and correctly, adopting good personal sanitation habits are measures to reduce the chances of getting infection.
  - (1) Wash your hands with soap and water frequently.
  - (2) Clean toys often and do not put them in the mouth.
  - (3) Don't go to crowded public areas to avoid infections.
  - (4) Go to see doctors as soon as possible while being sick and take days off to get more rests.
  - (5) Watch for the cleaning and ventilation of the house.
  - (6) Always wash hands before touching children.
  - (7) All adults and children should keep sanitation.
  - (8) Avoid close contact with people who are sick, especially for the pregnant women, newborns and young children.

More Information

1. WHO|Hand, Foot and Mouth Disease (HFMD)
2. USA CDC|Hand, Foot and Mouth Disease (HFMD)
3. USA CDC|Non-Polio Enterovirus

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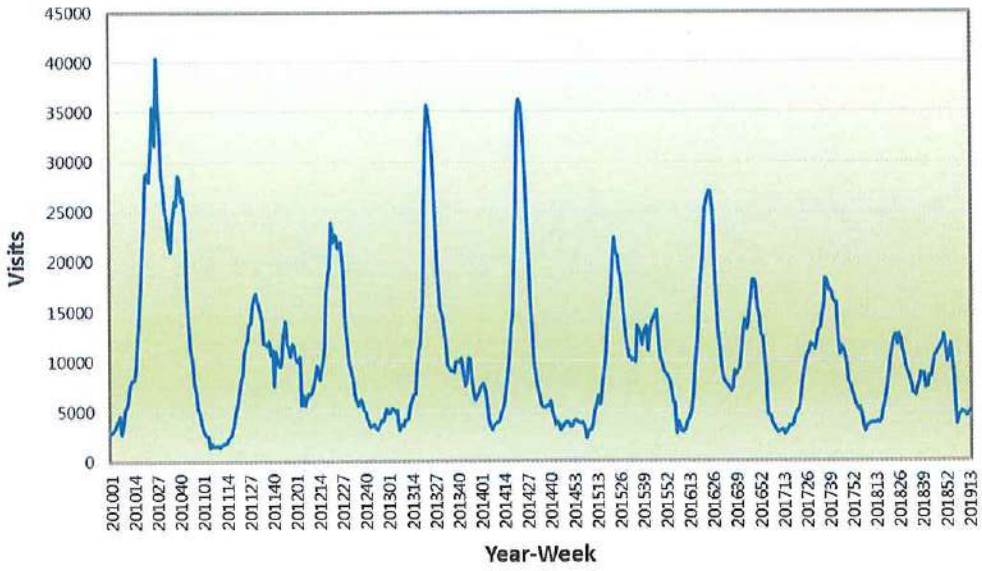

Figure 1: The number of weekly outpatient and emergency visits for enterovirus infection in Taiwan, 2010-2018

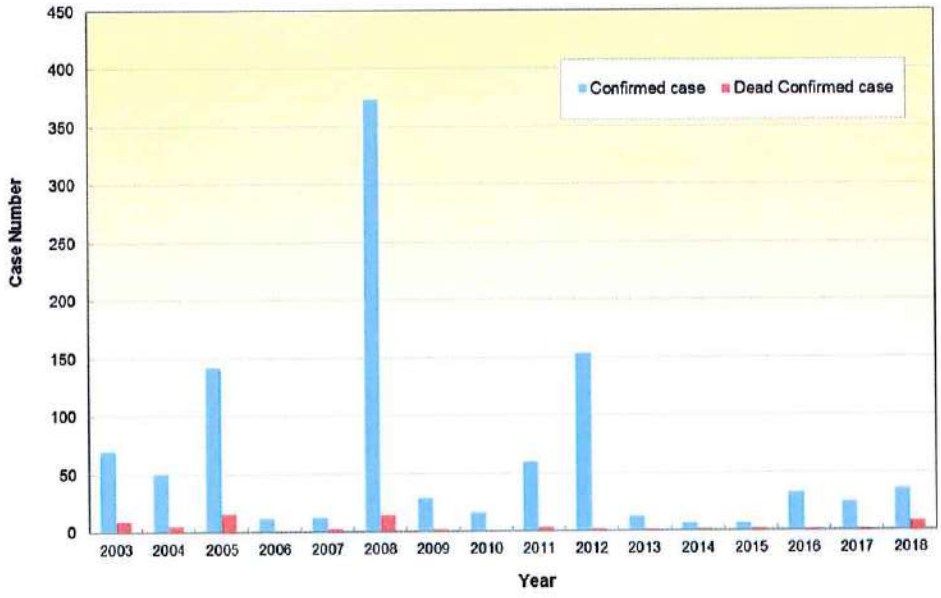

Figure 2: Volume of Confirmed Cases and Deaths of EVSC in Taiwan, 1998-2018

Resource from: Taiwan Centers for Disease Control
https://www.cdc.gov.tw/En/Category/ListContent/bg0g_VU_Ysrgkes_KRUDgQ?uaid=zRqpJ3zn3lI6Tc0LgD0Clw