

A Medical Mystery of Epidemic Proportions

Teacher Information



just add students™

Summary

Students explore a case study about a cholera epidemic and conduct laboratory tests to:

- Identify the disease-causing organism.
- Determine why people who have had cholera or been vaccinated do not have immunity.
- Determine how the new type of cholera-causing bacteria could have evolved.

They also consider courses of action that could be used to prevent the spread of cholera.

Core Concepts

- Diseases may be caused by bacterial pathogens.
- The immune system recognizes pathogens and produces specific antibodies to destroy pathogens
- Genetic changes in pathogens may allow them to escape immune system detection.
- Actions can be taken to prevent the spread of pathogens.

Time Required

Two 40-minute class periods + homework

Kit contains

- *Vibrio cholerae* Rapid-Test: 2 dip sticks, simulated “diarrhea” and “well water” samples, and instructions
- Antibody Test: simulated “O1 Antibody”, simulated “Known O1 Vc” and “Vc from Patient” samples, labeled droppers, Antibody Test strip, and instructions
- DNA Microarray Test: simulated microarray, simulated “O139 DNA”, labeled dropper, and instructions

Teacher Provides

- Safety goggles

Warning: Choking Hazard This Science Take-Out kit contains small parts. Do not allow children under the age of seven to have access to any kit components.

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Reusing *A Medical Mystery of Epidemic Proportions* kits

Teachers will need to instruct students on how to handle clean-up and return of the re-usable kit materials. For example, teachers might provide the following information for students:

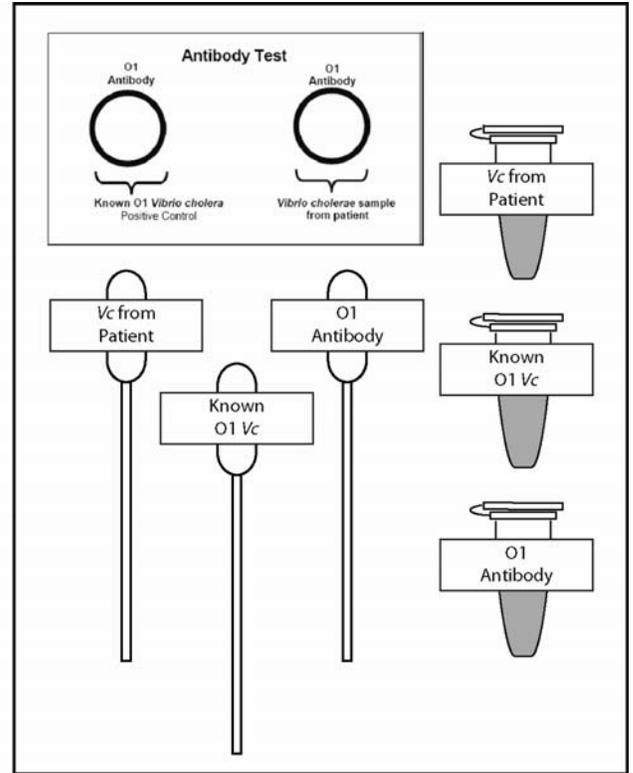
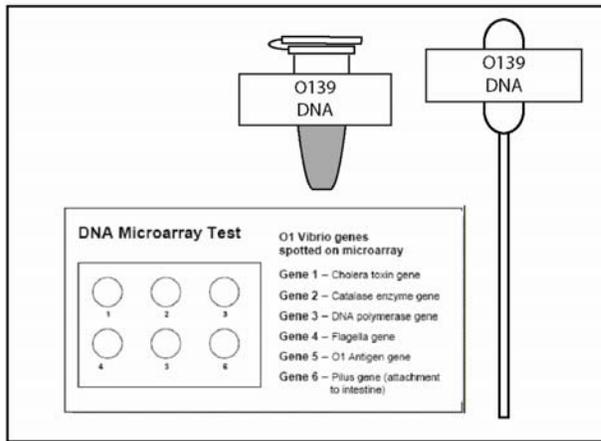
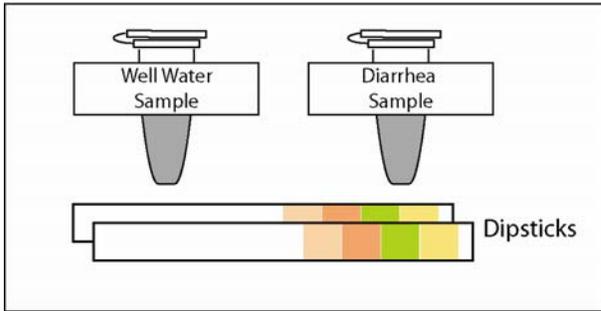
Discard	Rinse with water and dry with paper towel	Return to kit
<ul style="list-style-type: none"> • Used dipsticks • Used DNA Microarray Test card 	<ul style="list-style-type: none"> • Droppers • Antibody Test strip 	<ul style="list-style-type: none"> • All labeled microtubes • Labeled plastic bags • Droppers (rinsed) • Instruction cards for <i>Vibrio Cholerae</i> Rapid Test and Antibody Test* • Color graphic cards*

* Note: Consider laminating printed parts of the kits that will be reused.

Refills for *A Medical Mystery of Epidemic Proportions* kits are available at www.sciencetakeout.com. The **10 Kit Refill Pack** includes the following materials:

- 1 Quick Guide for refilling kit
- 6 graduated transfer pipets (teacher use only)
- 20 dipsticks
- 20 ml simulated “Well Water Sample”
- 20 ml simulated “Diarrhea Sample”
- 5 ml simulated “Vc from Patient”
- 5 ml simulated “Known O1 Vc”
- 10 ml simulated “O1 Antibody”
- 10 ml simulated “O139 DNA”
- 10 DNA Microarray Test cards

Kit Contents Quick Guide



Read these instructions before using Science Take-Out kits

Parental or Adult Supervision Required

This kit should be used only under the supervision of an adult who is committed to ensuring that the safety precautions below, and in the specific laboratory activity, are followed.

Safety Goggles and Gloves Strongly Recommended

We encourage students to adopt safe lab practices, and wear safety goggles and gloves when performing laboratory activities involving chemicals. Safety goggles and gloves are not provided in Science Take-Out kits. They may be purchased from a local hardware store or pharmacy.

Warning: Choking and Chemical Hazard

Science Take-Out kits contain small parts that could pose a choking hazard and chemicals that could be hazardous if ingested. Do not allow children under the age of seven to have access to any kit components. Material Safety Data Sheets (MSDS) provide specific safety information regarding the chemical contents of the kits. MSDS information for each kit is provided in the accompanying teacher instructions.

Chemicals Used in Science Take-Out Kits

Every effort has been made to reduce the use of hazardous chemicals in Science Take-Out kits. Most kits contain common household chemicals or chemicals that pose little or no risk.

General Safety Precautions

1. Work in a clean, uncluttered area. Cover the work area to protect the work surface.
2. Read and follow all instructions carefully.
3. Pay particular attention to following the specific safety precautions included in the kit activity instructions.
4. Goggles and gloves should be worn while performing experiments using chemicals.
5. Do not use the contents of this kit for any other purpose beyond those described in the kit instructions.
6. Do not leave experiment parts or kits where they could be used inappropriately by others.
7. Never taste or ingest any chemicals provided in the kit – they may be toxic.
8. Do not eat, drink, apply make-up or contact lenses while performing experiments.
9. Wash your hands before and after performing experiments.
10. Chemicals used in Science Take-Out experiments may stain or damage skin, clothing or work surfaces. If spills occur, wash the area immediately and thoroughly.
11. At the end of the experiment, return ALL kit components to the kit plastic bag. Dispose of the plastic bag and contents in your regular household trash.

No blood or body fluids from humans or animals are used in Science Take-Out kits. Chemical mixtures are substituted as simulations of these substances.

A Medical Mystery of Epidemic Proportions: *Teacher Answer Key*

Daphne's Blog - Sunday

I'm not sure my decision to be a Peace Corp volunteer was a good idea. I thought I was prepared for working in a village where extreme poverty and lack of transportation made obtaining food, water and medical care difficult. But, this has become really scary!

When I look out of my tent, I can see sick villagers curled up in the shade of a tree. They are waiting to be admitted to an already crowded hospital tent. One girl, weak and dehydrated, died of the severe diarrheal disease before she could get treatment.

We had a very heavy rainstorm here on Friday. I think that the rain's runoff carried human waste into the village's water supply. It's like the villagers are drinking poison. I'm afraid that this might be the beginning of a cholera epidemic. Here's what I found on the Internet about cholera.

Cholera is an intestinal infection caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*. It has a short incubation period, from less than one day to five days.

Vibrio cholerae bacteria produce a potent toxin that causes a large amount of watery diarrhea that can quickly lead to severe dehydration and death if treatment is not promptly given.

Cholera is usually transmitted through water or food that is contaminated by human feces containing *Vibrio cholerae* bacteria. Cholera outbreaks can occur in any part of the world where water supply, sanitation, food safety, and hygiene are inadequate.

1. What bacterial pathogen causes cholera?

Vibrio cholerae

2. How do these bacteria lead to the death of people who are not given prompt treatment?

The bacteria produce a potent toxin that causes a large amount of watery diarrhea that can lead to severe dehydration.

3. How is cholera spread from one person to another?

Cholera is spread through water or food that is contaminated with human feces containing Vibrio cholerae bacteria.

Part I: What is causing this disease outbreak?

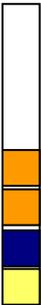
Daphne's Blog - Monday

An emergency medical team arrived and set up a hospital tent. They brought boxes of IV bags and antibiotics provided by American donors. Prompt treatment of diarrheal disease with an IV fluid is critical! The patients can die very quickly if IV's aren't started immediately to replace the fluids that victims have lost.

This epidemic is horrible! There are many new patients today. The hospital beds are just cots with a hubcap-sized hole cut in the middle. A bucket sits under the hole. Another bucket rests on the floor next to the victim's head. The patients are too weak to speak. Groans, sounds of vomit and diarrhea splashing into the buckets, and a horrible stench fill the crowded hospital. The hospital tent's 110 beds are full but the patients keep arriving.

There are many pathogens that could cause an outbreak of diarrheal disease. Luckily they have a *Vibrio cholerae* Rapid-Test Kit that can determine if this outbreak is caused by *Vibrio cholerae* bacteria. If this is a cholera outbreak, it will be important to take quick action to prevent the spread of *Vibrio cholerae* bacteria to other villages.

1. Use the *Vibrio cholerae* Rapid-Test Kit to test a sample of well water from the village and a diarrhea sample collected from a patient.
2. Record your laboratory observations by coloring or labeling the pictures of the dipsticks.
3. Record your conclusions based on the Rapid-Test results. Does the test show that *Vibrio cholerae* are present?

Well Water Sample	Diarrhea Sample	<i>Vibrio cholerae</i> Rapid-Test Kit Laboratory Observations
		Conclusions: <i>Both the well water sample and the diarrhea sample contain Vibrio cholerae bacteria. OR The villagers have cholera.</i>

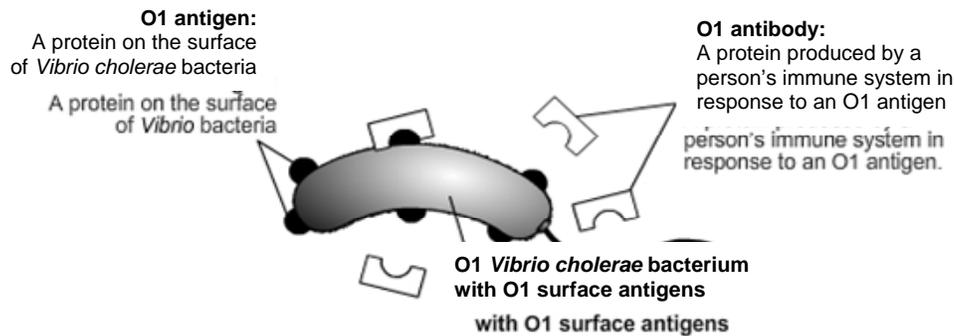
Part 2: Why are people who should be immune getting sick?

Daphne's Blog - Wednesday

Two members of the medical team have developed cholera. That shouldn't be happening because they both had cholera vaccinations. People who had cholera before are also getting sick. This is turning into a very scary medical mystery! Why

According to the doctors, only one type of *Vibrio cholerae* bacteria causes epidemic cholera – this is known as the O1 type. People who have been infected with cholera in the past and people who had cholera vaccinations should be immune to the O1 type of cholera. Their immune system should be making O1 antibodies that bind with and destroy the O1 type of *Vibrio cholerae*.

Type O1 *Vibrio cholerae* have O1 antigens on their surface. When a person is exposed to O1 *Vibrio cholerae*, their immune system produces specific O1 antibodies. These antibodies bind to O1 antigens and destroy the O1 *Vibrio cholerae*.



The emergency medical team is concerned that a new type of *Vibrio cholerae* bacteria might be causing this outbreak. If that's true, then the new type of *Vibrio cholerae* may not have O1 antigens. That would mean that the O1 antibodies cannot bind to and destroy the new type of *Vibrio cholerae*.

To determine if this is an O1 type or a new type of *Vibrio cholerae*, you can do an antibody test that uses O1 antibodies. If the O1 *Vibrio cholerae* bacteria are present in the patient sample, the O1 antibodies should bind with the O1 antigens on the bacteria surface. The binding will cause the *Vibrio cholerae* to clump together.

1. Which type of *Vibrio cholerae* bacteria is known to cause cholera epidemics?

O1 Type *Vibrio cholerae*

2. What is the difference between an O1 antigen and an O1 antibody?

An O1 antigen is a protein on the surface of an O1 *Vibrio cholerae* bacterium. An O1 antibody is a protein made by an infected person's immune system.

3. Explain why people who were infected by O1 *Vibrio cholerae* before should not get sick when they are exposed to this pathogen again.

They were exposed to the *Vibrio cholerae* before so their immune systems should be making O1 antibodies that destroy the *Vibrio cholerae*.

4. What results should you observe if O1 antibodies are mixed with O1 *Vibrio cholerae* bacteria?

There should be visible evidence of clumping.

5. Use the Antibody Test Kit to test the *Vibrio cholerae* sample from this cholera outbreak.

6. Record your laboratory observations indicating whether the samples clumped or did not clump.

7. Record your conclusions based on the results of the antibody testing.

Antibody Testing Laboratory Observations

<p>O1 Antibody solution</p>  <p>Known O1 <i>Vibrio cholerae</i> (Positive Control)</p>	<p>O1 Antibody solution</p>  <p><i>Vibrio cholerae</i> sample from patient</p>
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Conclusions:

The *Vibrio cholerae* sample from the patient does not have O1 antigens on its surface. This must be a new or different kind of *Vibrio cholerae*.

8. Why was it important to test a known sample of O1 *Vibrio cholerae* when using the antibody test kit?

This is a control to be certain that the O1 antibodies are working properly.

9. Explain why the adults who had cholera in the past or who had been vaccinated for O1 type *Vibrio cholerae* are not immune during this cholera epidemic.

Their O1 antibodies do not recognize this new pathogen OR the new kind of Vibrio does not have O1 antigens on its surface.

Part 3: How did O139 evolve?

Daphne's Blog - Friday

OK, one mystery has been solved. This cholera outbreak is caused by a **new** type of *Vibrio cholerae* called **O139**.

But now there is a new mystery. How did this new O139 *Vibrio cholerae* evolve? The scientists I've been talking to have two hypotheses.

Hypothesis 1: The new O139 *Vibrio cholerae* evolved from a non-pathogenic (harmless) type of *Vibrio cholerae* that picked up a gene for the cholera toxin. If that happened the harmless type could become a "killer" *Vibrio cholerae* that is not recognized by O1 antibodies.

Hypothesis 2: The new O139 *Vibrio cholerae* evolved from an O1 *Vibrio cholerae* that mutated and lost the ability to make the O1 antigen. If that happened, people's O1 antibodies also wouldn't be able to recognize the new O139 *Vibrio cholerae*.

To see which hypothesis is correct, the doctors have sent a sample of the new O139 *Vibrio cholerae* to the CDC (Center for Disease Control). Researchers at the CDC will use a DNA test called a "microarray" to compare the genes of the O1 *Vibrio cholerae* with the genes of the new O139 *Vibrio cholerae*.

1. Observe diagrams A and B on the colored graphic sheet in your lab kit.

- Which diagram best illustrates Hypothesis 1? **A**

Explain your answer:

Because the first Vibrio was harmless and diagram A shows it getting a toxin gene.

- Which diagram best illustrates Hypothesis 2? **B**

Explain your answer:

Because the first Vibrio was an O1 type and diagram B shows it losing the gene for the O1 surface antigen.

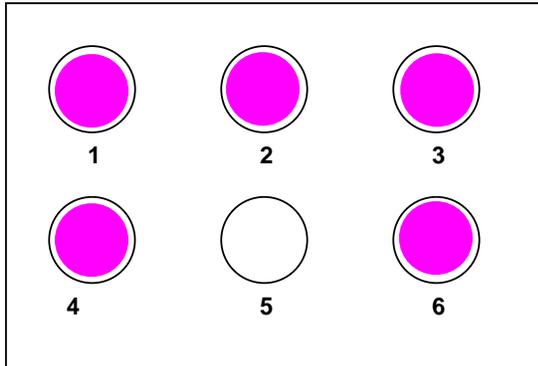
2. Your lab kit contains a simulated DNA microarray has been spotted with DNA from many different O1 *Vibrio cholerae* genes. Your kit also contains a tube of simulated DNA isolated from the O139 *Vibrio cholerae*.

3. Follow the instructions in the DNA Microarray Test Kit to test the O139 DNA sample.

4. Record your laboratory observations below by coloring in the DNA spots that are pink.

5. Record your conclusions from the microarray testing.

DNA Microarray Laboratory Observations



O1 *Vibrio cholerae* genes spotted on the microarray:

Gene 1 – Cholera toxin gene

Gene 2 – Catalase enzyme gene

Gene 3 – DNA polymerase gene

Gene 4 – Flagella gene

Gene 5 – O1 Antigen gene

Gene 6 – Pilus gene (attachment to intestine)

Conclusions:

O139 does not have an O1 antigen gene. All of its other genes are the same as the O1 genes.

6. Which hypothesis is supported by the results of your DNA microarray analysis?

- Hypothesis 1: O139 evolved from a harmless *Vibrio cholerae* that picked up a gene for the cholera toxin.
- Hypothesis 2: O139 evolved from an O1 *Vibrio cholerae* that lost the gene for the O1 surface antigen.

Hypothesis: 2

Support your answer with evidence from the DNA microarray testing.

Most of the O139 genes are like the O1 genes. The O139 DNA did not contain the gene for the O1 surface antigen.

7. Why is the evolution of a new type of *Vibrio cholerae* a serious health threat?

Because people who had cholera before or who were vaccinated can spread this disease to others. OR Because more people can get this type of cholera because they do not make antibodies against it. OR Because the current cholera vaccine won't protect people from O139.

Part 4: What can be done to prevent cholera epidemics?

Daphne's Blog - Sunday

The medical team says that we need to take action to prevent additional cases of cholera. They've asked me to work with people who live in this village and nearby villages to make them aware of what they could do to prevent the spread of the O139 *Vibrio cholerae*.

The immediate solutions seem so simple. Encourage villagers to use the temporary supply of clean water provided by the medical team. Drill wells that would provide clean water. Build latrines so that villagers wouldn't use their fields as open-air toilets. But these options are too expensive unless the government steps in.

Hopefully, in the future, scientists will develop a new vaccine that protects people from the O139 *Vibrio cholerae*.

1. What actions should villagers take to prevent O139 type *Vibrio cholerae* from causing future cholera epidemics?

Student answers will vary but may include:

- ***Keep the water from getting polluted.***
- ***Do not drink contaminated food or water.***
- ***Drill new wells to get clean water.***
- ***Build latrines.***
- ***Make a new vaccine that works for O139.***

2. Why are implementing these actions difficult in many areas of the world?

Student answers but may include: poverty, lack of medical supplies, lack of food, lack of water, lack of education may prevent taking needed actions.

MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name (as printed on the label): "Well Water Sample", "Diarrhea Sample" and "O139 DNA"

Product identity: Hydrion Buffer Salt pH 10

Distributor: Microessential Laboratory Inc. PO Box 10824, 4224 Avenue H, Brooklyn, NY 11210

Telephone number for information: (718)338-3618 Medical emergency phone number (Chemtrec): (800) 424-9300

Date of this MSDS: 12/31/08

2. COMPOSITION/INFORMATION ON INGREDIENTS

Product	Ingredients	CAS Numbers	% Weight/Volume (balance is water)
pH 10 buffer	Sodium carbonate	497-19-8	0.25%
	Sodium bicarbonate	144-55-8	0.15%

For all the ingredients OSHA PEL: TWA – none estab. STEL – none estab.
ACGIH TLV: TWA – none estab. STEL – none estab.
NIOSH REL: TWA – none estab. STEL – none estab.
NIOSH ILDH: none estab.

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW

Do not ingest. Avoid skin and eye contact. Avoid exposure to vapor or mists.

Potential Health Effects

EYES: May cause irritation. SKIN: May cause irritation. INHALATION: n/a
INGESTION: May cause gastrointestinal discomfort and mouth burns.

4. FIRST AID MEASURES

EYES - Flush with water for at least 15 minutes, raising and lowering eyelids occasionally. Get medical attention if irritation persists.

SKIN - Thoroughly wash exposed area for at least 15 minutes. Remove contaminated clothing. Launder contaminated clothing before reuse. Get medical attention if irritation persists.

INGESTION - Do not induce vomiting. If swallowed, if conscious, give plenty of water immediately and call a physician or poison control center. Never give anything by mouth to an unconscious person.

5. FIRE FIGHTING MEASURES

NFPA Rating: Health: 1 Fire: 0 Reactivity: 0

Extinguisher Media: Any means suitable for extinguishing surrounding fire

Special Firefighting Procedures: Firefighters should wear full protective equipment and NIOSH approved self-contained breathing apparatus.

Unusual Fire and Explosion Hazards: No data available

6. SPILL OR LEAK PROCEDURES

Ventilate area of spill. Clean-up personnel should wear proper protective equipment and clothing. Absorb material with suitable absorbent and containerize for disposal.

7. HANDLING AND STORAGE

Store in a cool dry place. This Material is not considered hazardous. Handle using safe laboratory practices.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Respiratory Protection: n/a

Ventilation: Local Exhaust: Preferred Mechanical(General): Acceptable Special: No Other: No

Protective Gloves: Natural rubber, Neoprene, PVC or equivalent.

Eye Protection: Splash proof chemical safety goggles should be worn.

Other Protective Clothing or Equipment: Lab coat, apron, eye wash, safety shower.

9. PHYSICAL AND CHEMICAL PROPERTIES

Melting Point: ~0°C

Boiling Point: ~100°C

Vapor Pressure: information not available

Vapor Density: information not available

Specific Gravity (H₂O=1): ~1

Percent Volatile by Volume: >99

Evaporation Rate: information not available

Solubility in Water: soluble

Appearance and Odor: Clear colorless liquid

10. STABILITY AND REACTIVITY

Stability: Stable

Materials to Avoid: strong acids and bases

Hazardous Decomposition Products: none known

Hazardous Polymerization: will not occur

11. TOXICOLOGICAL INFORMATION

Ingredient	Toxicity (oral-rat) LD ₅₀
Sodium carbonate	4090 mg/kg
Sodium bicarbonate	4220 mg.kg

Effects of Overexposure (for all pH buffers):

Acute: Essentially non-hazardous. Possible irritation of eyes/skin/stomach

Chronic: None known.

Conditions aggravated/Target organs: none known

Target Organs: Eyes, skin, and gastrointestinal tract.

Primary Route(s) of Entry: Ingestion or skin contact.

12. ECOLOGICAL INFORMATION

No ecological data available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods: Dispose in accordance with all applicable Federal, State and Local regulations.

Always contact a permitted waste disposer (TSD) to assure compliance.

14. TRANSPORTATION INFORMATION

D.O.T. SHIPPING NAME: Not regulated

15. REGULATORY INFORMATION

EPA regulations: RCRA Hazardous waste number (40 CFR 261.33) – not listed

RCRS Hazardous waste classification (40 CFR 261) – not classified

SARA Toxic Chemical (40 CFR 372.65) – not listed

SARA EHS (Extremely Hazardous Substance (40 CFR 355) – not listed

OSHA regulations: Air Contaminant (29 CFR 1910.1000) – not listed

16. ADDITIONAL INFORMATION

The information provided in this Material Safety Data Sheet represents data from the manufacturer and/or vendor and is accurate to the best of our knowledge. By providing this information, Science Take-Out LLC makes no guarantee or warranty, expressed or implied, concerning the safe use, storage, handling, precautions, and/or disposal of the products covered or the accuracy of the information contained in this fact sheet. It is the responsibility of the user to comply with local, state, and federal laws and regulations concerning the safe use, storage, handling, precautions, and/or disposal of products covered in this fact sheet.

MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name (as printed on the label):

"Vc from Patient" (simulated), "Known O1 Vc" (simulated), "O1 Antibody"(simulated)

Product identity: Food coloring – < 0.1% Inorganic salts – 8-16%
Water – 84-92%

Manufacturer: Science Take-Out, LLC
P.O. Box 205
Pittsford, NY 14534

Telephone number for information: (585)764-5400
Preparation date of this MSDS: 10/5/08
Medical emergency phone number (Chemtrec): (800) 424-9300

2. COMPOSITION/INFORMATION ON INGREDIENTS

This product contains no hazardous materials as defined by the OSHA Hazards Communications Standard

Chemical Ingredients: Red food coloring (1%) Chemical Name: N/A

CAS Number: N/A Formula: N/A Synonyms: N/A

Principle Hazardous Components: No Data

TLV and PEL units: No Data OSHA-PEL 10ppm (TWA): No Data

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW

Avoid skin and eye contact.

Potential Health Effects EYES: May cause irritation. SKIN: May cause irritation.

4. FIRST AID MEASURES

EYES - Flush with water for at least 15 minutes, raising and lowering eyelids occasionally. Get medical attention if irritation persists.

SKIN - Thoroughly wash exposed area.

5. FIRE FIGHTING MEASURES

 No data available

6. SPILL OR LEAK PROCEDURES

Wear proper eye and skin protection. Mop/wipe spill area. Rinse with water.

7. HANDLING AND STORAGE

 Avoid eye and skin contact

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Respiratory Protection: N/A Ventilation: N/A

Protective Gloves: Natural rubber, Neoprene, PVC or equivalent.

Eye Protection: Splash proof chemical safety goggles should be worn.

Other Protective Clothing or Equipment: None

9. PHYSICAL AND CHEMICAL PROPERTIES

Molecular Weight: No data Melting Point: N/A

Boiling Point: No data Vapor Pressure: No data
Vapor Density (Air=1): No data Specific Gravity (H₂O=1): No data
Percent Volatile by Volume: No data Evaporation Rate (BuAc=1): No data
Solubility in Water: Soluble
Appearance and Odor: "Vc from Patient", "Known O1 Vc" = clear colorless liquid; "O1 Antibodies" = blue liquid

10. STABILITY AND REACTIVITY

Stability: Stable Conditions to Avoid: No data
Incompatibility (Materials to Avoid): None Hazardous Decomposition Products: No Data
Hazardous Polymerization: Will not occur

11. TOXICOLOGICAL INFORMATION

Toxicity Data: No data Effects of Overexposure: See section 3
Target Organs: Eyes and skin Primary Route(s) of Entry: Eye or skin contact.

12. ECOLOGICAL INFORMATION No data

13. DISPOSAL CONSIDERATIONS Can be disposed of in trash or down the sink.

14. TRANSPORTATION INFORMATION D.O.T. SHIPPING NAME: N/A

15. REGULATORY INFORMATION N/A

16. ADDITIONAL INFORMATION

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