A PRACTICAL METHOD FOR RAPID ASSESSMENT OF THE BACTERIAL QUALITY OF WATER

A FIELD-BASED GUIDE

UNITED NATIONS HUMAN SETTLEMENTS PROGRAMME

UN HABITAT
FOR A BETTER URBAN FUTURE

A Practical Method for Rapid Assessment of the Bacterial Quality of Water

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HS Number: HS/179/10E

ACKNOWLEDGEMENTS:

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Printer: UNON, Publishing Services Section, Nairobi, ISO 14001:2004-certified.

Cover Photo: *Taking a water sample from an open well in Dar es Salaam, Tanzania. Photo © Robert H. Metcalf*

INTRODUCTION

Welcome! The instructions in this booklet will teach you and your community how to test drinking water.

It is important for communities to know if their drinking water sources are safe or contaminated because people can get sick if they drink water contaminated with disease-causing microbes, such as bacteria and viruses. Diseases such as typhoid, cholera, and dysentery are caused when these microbes infect the intestinal tract, and are shed by the billions in faeces from sick people. When these microbes get into drinking water they can infect other people and cause more disease.

Until a few years ago, methods to test for microbial contamination in water required a well-equipped laboratory with electricity, incubators and sterilization equipment. Recent advances in research showed that the indicator bacterium Escherichia coli could use specific nutrients that other bacteria cannot use. Based on this discovery, a new generation of tests for *E. coli* was introduced. Because the only requirement to perform these tests is to add the water source, the tests can be performed in any setting with minimal training. Today, these tests are used in the most advanced water and food testing labs in developed countries, but they can also be used in remote villages. In both settings, the goal is to detect the presence of *E. coli*.

Figure 2: *Sampling water from a shallow well in Nyakach, Kenya. Photo © Robert H. Metcalf*

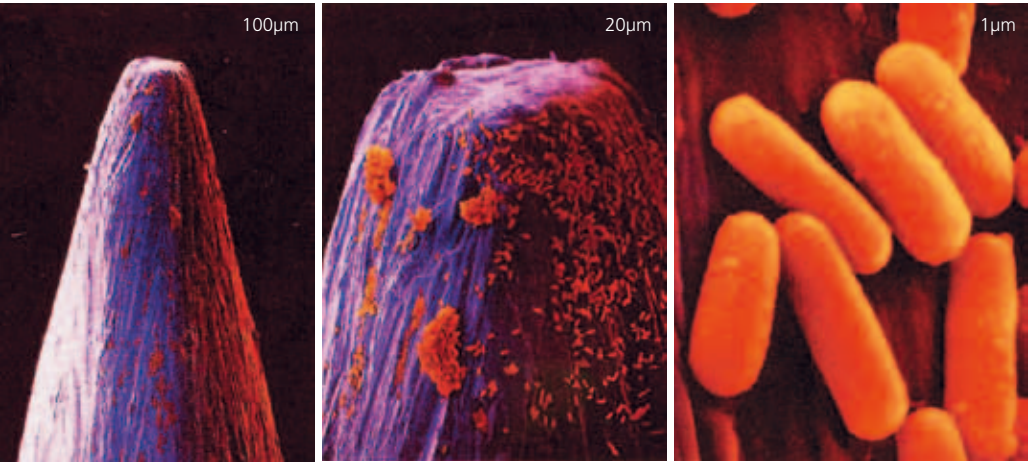


Figure 3: *Bacteria on the tip of a pin. Photo © Microbiology - Principles and Explorations, 4th Ed., John Wiley & Sons, Hoboken, NJ.*

E. COLI AS AN INDICATOR

It is not practical or possible to test drinking water for the dozens of microbes that can cause disease. Instead, water is tested for the presence of a bacterium that indicates recent fecal contamination. This indicator bacterium has the name of *Escherichia coli*, commonly abbreviated as *E. coli.*

The reasons *E. coli* has been the best indicator of recent fecal pollution of water for over a century are:

1) *E. coli* is always present in the feces of humans and other mammals in large numbers, whether one is healthy or sick (approximately one hundred million to one billion *E. coli* cells per gram of human feces!)

2) It does not grow in the environment, such as on plants, in soil or in water

3) *E. coli* slowly dies when shed in feces, but it survives in water at least as long as the bacteria that cause typhoid fever, cholera, and dysentery

4) It is relatively easy to detect

The presence of *E. coli* in drinking water, therefore, indicates recent fecal contamination, and the possibility that disease causing microbes may also be in the water.

WATER SAMPLING

PARTICIPATORY SAMPLING

In the development context, any sampling of drinking water supplies needs to take into account complex water supply arrangements, especially in urban areas. A range of alternative water sources may be in use, including point sources and vended water. In places where water supplies are community managed, as is often the case in slums, peri-urban and rural areas, treatment and testing of water is seldom carried out. At the same time, it is often these supplies that present the greatest water quality problem. A lot can be gained by taking a participatory approach, involving communities in sampling, analysis and information exchange. Doing water testing in these settings should be mainly geared towards providing a supportive role to enhance community management.

THE SAMPLING NETWORK

When designing a water sampling network, it is very important to identify the critical sampling points within a water supply system. Attention should be given to the most widely used water sources and sampling should be done precisely where water is taken for use. In the initial stage it can be useful to conduct a town-wide survey to map water sources in use and also to identify the most polluted sources. The sampling network should also aim to identify where contamination occurs so that appropriate mitigation measures can be introduced. For instance, sampling at various locations along a stream can be useful in order to assess where contaminants enter the river and subsequently track contaminants back to their source.

It is also highly useful to conduct sampling of household storage containers. Evidence suggests that extensive contamination occurs when water is transported and stored in the household and the sampling network should aim to identify whether this occurs. Households should be involved in the testing to visually observe and be informed about the quality of their water. When sampling storage containers, water should be carefully poured into the collection container using the device normally used to scoop water.

Generally, sampling at sites where water quality is expected to vary considerably should be done frequently in order to capture these changes. Sampling at sites where quality remains relatively constant can be done with greater intervals. If no information is available, or you are setting up a new monitoring programme, sampling should be done at frequent intervals in the beginning until the variability in quality is established. The sampling intervals can later be adjusted.

HOW TO USE THE WHIRL-PAK

1. Label the bag with sample information, such as location and date/time
2. Carefully tear off the top plastic section along the perforation. The inside of the Whirl-Pak is sterile, and you should be careful not to contaminate it with your fingers
3. Open the Whirl-Pak by pulling away on the two white tabs in the top center of the bag
4. Collect a water sample – running water into the Whirl-Pak, or by dipping the Whirl-Pak into a water source (and not your hands with it!), or by using the sterile plastic pipette to collect water and add to the Whirl-Pak. Fill the Whirl-Pak to a little above the 100 ml level
5. Once the sample has been collected, pull the ends of the wires together to close the bag. Then whirl it three complete revolutions to seal it

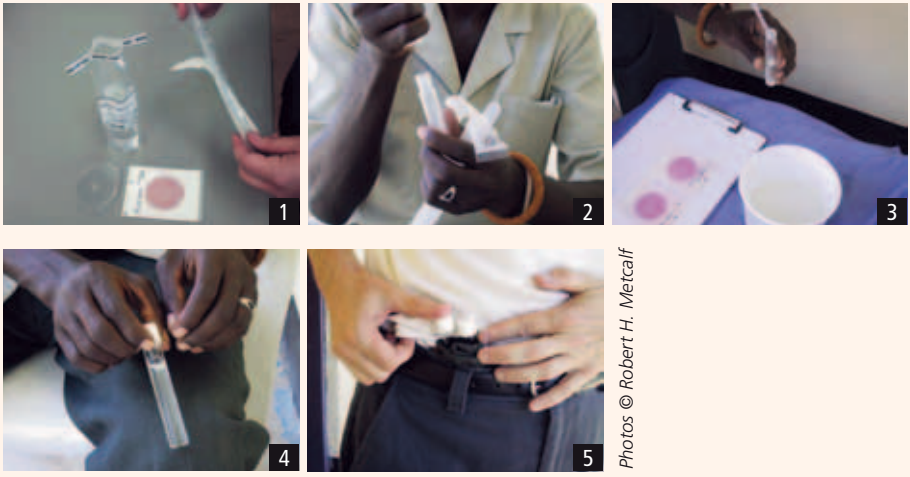
You should process the sample within 6 hours. Most of the time you could collect the sample and do the inoculations right away.

TESTING PROCEDURES

The two tests explained in this booklet are the IDEXX Colilert Presence/Absence test and the 3M Petrifilm E.coli/Coliform Count Plate test. The Colilert test is performed in a glass tube that contains a dried nutrient powder for *E. coli*. The test indicates presence/absence of coliform and *E. coli* bacteria in a 10 ml water sample. The Petrifilm is used to enumerate individual *E. coli* and coliform bacteria in 1 ml of water. The test is a flat, 7.5 x 10 cm rectangle composed of a bottom layer coated with sterile dried nutrients to support bacterial growth. A white foam layer around the growth area encapsulates the water sample.

HOW TO DO THE COLILERT TEST

1. Carefully remove a sterile pipette from its package
2. Carefully remove the cap of the Colilert tube so as not to contaminate it.
3. Add 10 ml of the water sample to the tube – to the mark on the tube.
4. Replace the cap. Mix, by inverting the tube several times, to dissolve the nutrients. The sample will be clear.
5. Place the pipette in its package to save it for inoculating a Petrifilm with this same water sample.
6. Incubate the tube at body temperature – 35ºC, to promote good bacterial growth. Tubes can be place in a small sack, or sock, and held close to the body – and slept on at night.
7. Examine the tubes after incubation for up to 24 hr. Results are often evident in 10-18 hours (10 hr with heavy contamination, 18 hr with lesser contamination). If the tube turns yellow, remember to shine on it with the UV light to look for presence of ***E. coli***.



Photos © Robert H. Metcalf

RESULTS OF THE COLILERT TEST

There are three possible results:

1. If the tube is clear, no coliforms are present.

2. If the tube is yellow, but there is no fluorescence under long-wave UV light, coliform bacteria other than *E. coli* are present. These are likely to come from the environment and do not have public health significance.

3. If the tube is yellow and fluoresces blue when a long-wave UV light shines on it, *E. coli* was present in the water sample, and the water poses a substantial health risk.

For complete description of how to use and interpret the Colilert Presence/ Absence test, please see **www.idexx.com/water**

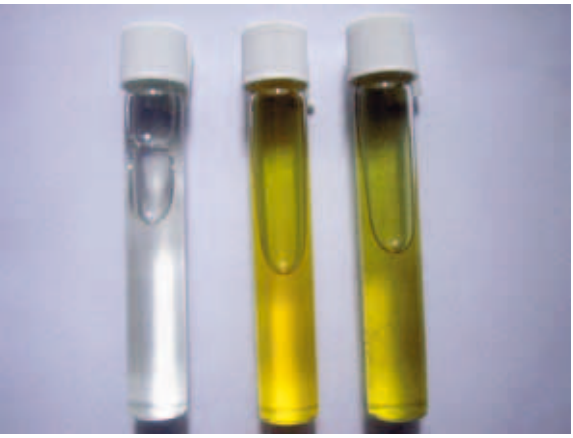


Figure 4: *Colilert tubes after incubation. The clear tube on the left has no coliforms; the two tubes on the right have some type of coliform bacteria. Photo © Robert H. Metcalf*

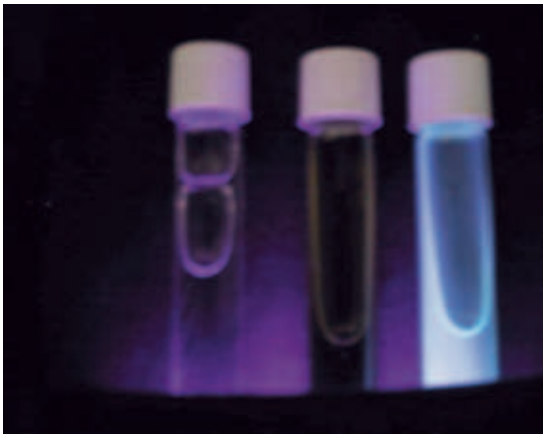


Figure 5: *The three Colilert tubes after incubation with a UV light shining on them. The center tube does not fluoresce and thus has only environmental coliforms. The tube on the right fluoresces, indicating the presence of E. coli. Photo © Robert H. Metcalf*

HOW TO DO THE PETRIFILM TEST

1. Place the ***E. coli*** Count Petrifilm on a flat surface. On the white part, write the location, date and time the sample is tested.
2. Lift top film and dispense 1 ml of sample onto the center of the red, dried nutrients. Do not touch the surface of the Petrifilm.
3. Slowly roll the top film down onto the sample to prevent entrapment of air bubbles. Do not let the top film drop.
4. Distribute the sample evenly within the circular area using a gentle downward pressure on the center of the plastic spreader (flat side down). Do not slide spreader across the film. Remove spreader and leave plate undisturbed for one minute for gel to solidify.
5. Incubate plates with clear side up at body temperature for up to 24 hours. You can stack up to 10 Petrifilms. Where there is no incubator, you can place the Petrifilms between two pieces of firm cardboard, securing them with rubber bands, and incubating the stack close to the body during the day, sleeping on them at night. Blue colonies are often visible in 10 hr, which then grow larger in size and produce gas bubbles with further incubation.

1


2


3


4


5

Photos © Robert H. Metcalf

RESULTS OF THE PETRIFILM TEST

The results can give the following:

1. *E. coli* colonies will appear blue with gas bubbles. One or more *E. coli* colonies signifies heavily contaminated water, which should be treated before drinking.

2. Non *E. coli* coliform colonies will be red with a gas bubble.

3. Non-coliform Gram negative bacteria form red colonies without a gas bubble.

Do not count colonies on the white foam surrounding the red growth area since they are removed from the selective influence of the medium.

Very high concentrations of *E. coli* will cause the growth area to turn a bluish color with individual colonies too tiny to distinguish. Very high concentrations of non *E. coli* coliforms will cause the growth area a dark reddish color with individual colonies too tiny to distinguish. If this occurs further dilution of the sample is required to obtain a more accurate count.

For the complete description of how to use and interpret the *E. coli* Count Petrifilm, see **www.3m.com/microbiology**

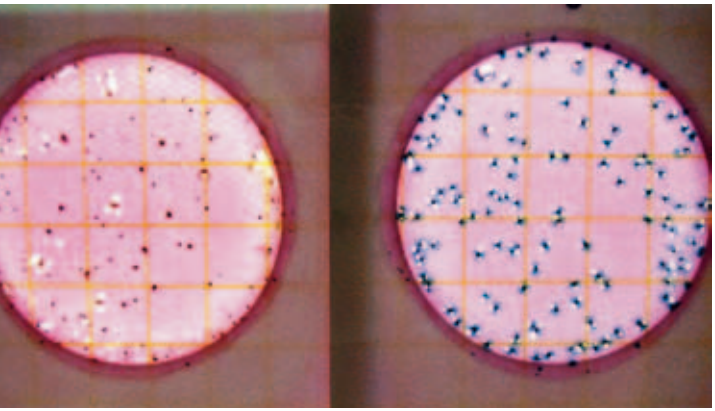


Figure 6: *The Petrifilm on the left has only bacteria from the environment. The Petrifilm on the right contains E. coli. Photo © Robert H. Metcalf*

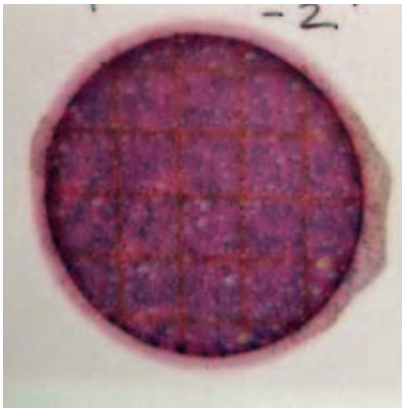


Figure 7: *A Petrifilm with very high concentrations of E. coli. Individual conlonies are too tiny to distinguish. Photo © Robert H. Metcalf*

INTERPRETING, RECORDING AND PRESENTING THE RESULTS

The Colilert and Petrifilm tests correlate with the relative risk of disease from drinking-water (WHO Guidelines for Drinking Water, 2nd Edition). Table 1 below shows how this risk assessment is done. If the Colilert test does not fluoresce when a UV light shines on it and there are no blue colonies with gas bubbles on the Petrifilm, the risk of disease is low. If the Colilert test fluoresces and the Petrifilm remains clear, the risk is moderate. If the Colilert test fluoresces and between 1 and 10 blue colonies with gas appear on the Petrifilm, the risk is high. Finally, if the Colilert test fluoresces and Petrifilm contains more than 10 blue colonies with gas, the risk is very high.

Table 1: Risk assessment of water sources

Risk level	*E. coli*/sample	Colilert fluorescence	Petrifilm # Blue&gas
Low	< 1/10 ml	-	0
Moderate	1-10/10 ml	+	0
High	1-10/ml	+	1-10
Very High	>10/ml	+	>10

When conducting water testing, large amounts of data are often generated. A good record of the results makes analysis and presentation much easier. Table 2 below shows how the results of the water testing can be recorded.

Table 2: Example data recording sheet

Test #	Date/time	Location	Water source	Colilert yellow/ clear	Did the Colilert tube fluoresce?	# blue & gas on Petrifilm	Risk of disease
1	23.03.2008	Central Market	Spring (improved)	yellow	yes	8	High

Additional information can include:

- Sanitary characteristics of area surrounding water source (sanitary score card)
- GPS readings
- Approximate population served
- Recommended actions
- Recommended retesting schedule
- Photo of water source

A powerful way of analyzing and presenting water quality results in real-time, if combined with geo-referencing information, is on a Geographic Information System (GIS). An example of this is the display of data through UN-HABITAT's partnership with Google.org in the h2.0 Information Services to Inform and Empower on **www.h20initiative.org.**

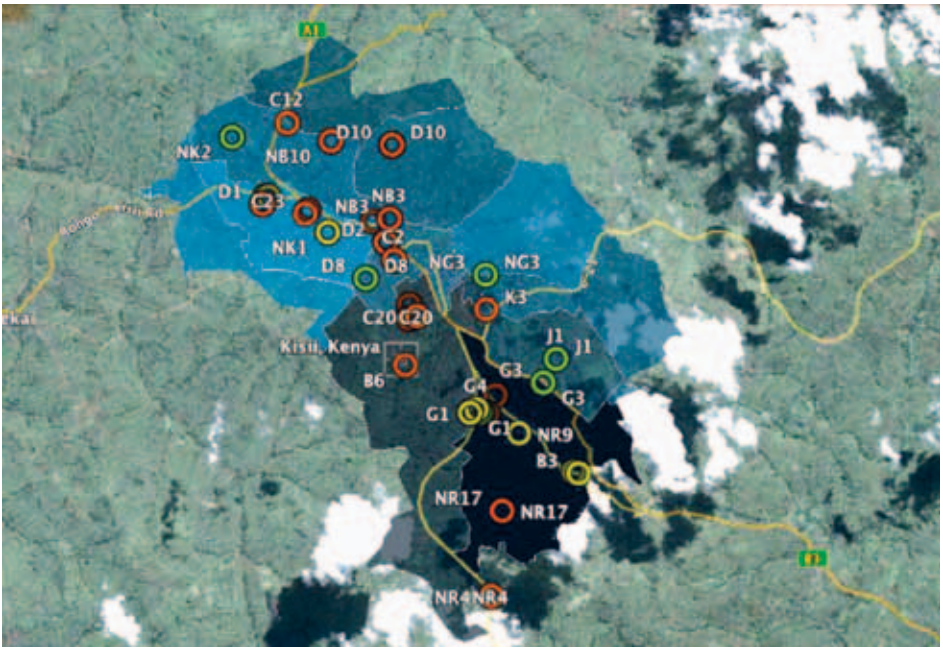


Figure 8: *Geo-referenced water quality data collected using the tests in Kisii (July 2010) and represented on an online mapping tool (green = low risk, yellow = moderate risk, orange = high risk). Photo © 2010 Google*

ANNEX I

INFORMATION FOR ADVANCED USERS

COLILERT PRESENCE/ABSENCE

The Colilert Presence/Absence test for water is a defined substrate medium, not containing organic sources of nitrogen and with only two carbon sources for bacteria to attack to obtain energy:

1. ONPG (Ortho-nitro-phenol-beta D-Galactopyranoside). Coliform bacteria can be induced to produce the beta-galactosidase enzyme, which breaks the bond between the indicator part, ONP, and the sugar, G (galactopyranoside). ONPG is colorless. ONP, however, has a bright yellow color.

2. MUG (4-methyl-umbelliferone-beta-D-Glucuronide). Among the coliform bacteria, only *E. coli* produces the constitutive enzyme beta-glucuronidase, which hydrolyzes the bond between the indicator part, MU (methylumbelliferone), and the sugar, G (glucuronide).The glucuronide is metabolized to enable growth of *E. coli*. MUG is colorless. MU, however, fluoresces blue when a long wave UV light (340 nm) shines on it.

The Colilert test has been developed to detect total coliform bacteria and *E. coli* simultaneously in potable and other waters within 24 hours.

E. COLI COUNT PETRIFILM

The *E. coli* Count Petrifilm is a reliable, sample-ready medium system for enumerating *E. coli* and coliforms. It is regularly used in the food industry to sample meat, seafood and poultry. *E. coli* Count Petrifilms contain:

- Violet red bile nutrients, which includes lactose. The bile salts and crystal violet in the medium inhibit Gram positive bacteria.
- A cold water soluble gelling agent
- A glucuronidase indicator (BCIG, 5-bromo-4 chloro-3 indolyl-beta D Glucuronide) to identify *E. coli* (the same enzyme which hydrolyzes MUG in the Colilert test)
- A tetrazolium indicator which Gram negative bacteria reduce to a red color to enhance colony visualization

All coliform bacteria ferment lactose to produce gas bubbles. The bubbles are trapped around the coliform colony. This will distinguish coliform bacteria from other Gram negative bacteria which do not produce gas bubbles from lactose.

In addition, glucuronidase, produced by most *E. coli*, will hydrolyze the glucuronide from BCIG. The BCI produces a blue precipitate around the colony allowing visual identification of *E. coli*, distinguishing it from non *E. coli* coliform colonies which are red with gas bubbles.

ANNEX II

SUPPLIERS OF EQUIPMENT

Below is a list of potential suppliers of equipment necessary to conduct the tests explained in this booklet. The list is not exhaustive and does not imply an endorsement of any supplier above another.

COLILERT PRESENCE/ABSENCE TEST

IDEXX Laboratories, Inc.
One IDEXX Drive, Westbrook,
Maine 04092 USA
Tel: +1 207-556-4496
or +1 800-321-0207
www.idexx.com/water

E. COLI COUNT PLATE PETRIFILM

3M Microbiology
St. Paul, MN 55144-1000, USA
Tel: +1 800-328-6553
www.3M.com/microbiology

4 OZ/100ML STAND-UP WHIRL-PAK

Nasco
901 Janesville Avenue
Fort Atkinson, Wisconsin,
53538-0910, USA
Tel: +1 920-563-2446

STERILE PLASTIC PIPETTE, INDIVIDUALLY WRAPPED, GRADUATED TO 1 ML (3.5 ML CAPACITY, INCL. BULB)

Can be obtained from various suppliers of laboratory products including:

Evergreen Scientific
2300 East 49th Street
PO Box 58248
Los Angeles, CA 90058, USA
Tel: +1 323-583-1331
E-mail: info@evergreensci.com

LASEC
52 Old Mill Road, Ndabeni, 7405
PO Box 2110, Cape Town, 8000
South Africa
Tel: +27 21 531 7504
E-mail: sales@lasec.co.za

DEHTEQ – Neway Technologies Ltd.
Nairobi, Kenya
Tel: +254 (20) 311225
E-mail: kahociojn@yahoo.com

LONG-WAVE HANDHELD UV LIGHT, BATTERY OPERATED

Can be obtained by various suppliers of equipment to the audio and lighting industry including:

Audiosure
Johannesburg, South Africa
Tel: +27 (11) 790 4600
E-mail: sales@audiosure.co.za

SeaCorals LLC
PO Box 3
Lake Junaluska, NC 28745
USA
www.seacorals.net

Musician's Friend, Inc.
PO Box 4370
Medford, OR 97501-0168
http://www.musiciansfriend.com

DISCLAMIER

This water testing methodology aims to indicate levels of disease risk as a result of fecal contamination. It is not intended to replace standard methods for water testing or any Government approved water testing procedures.

Figure 9: Community members displaying water testing results in Nkomo village, Shinyanga Region, Tanzania. Photo © Robert H. Metcalf

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