IFU

Instructions for Use

Cat. no. K226	C Diff Banana Broth™, 16x100mm Tube, 10ml	20 tubes/box
Cat. no. 972029	Flocked swab, standard-tip, rigid-shaft, for general use, 152mm ABS plastic handle with 20mm flocked nylon tip, breakpoint of 30mm, sterile**	100 swabs/pack
** Item sold separately		

INTENDED USE

Hardy Diagnostics C Diff Banana Broth™ is recommended as a selective enrichment broth for the culture and recovery of *C. difficile* from environmental samples.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Approximately 1.7 million hospitalized patients develop nosocomial infections annually while being treated for entirely unrelated conditions; more than 98,000 (one in 17) of these patients will die due to complications from acquired infections.[5] Consequently, healthcare-associated infections (HAI) are a major cause of morbidity and mortality around the world, posing an enormous financial and physical burden on the global health care system.

In the U.S., the Centers for Disease Control and Prevention state that *Clostridioides difficile* (formerly *Clostridium difficile*) infection (CDI) surveillance is a core component of the CDC's Emerging Infections Program, Healthcare-Associated Infections Community Interface (HAIC).[3] *C. difficile* causes diarrhea linked to approximately 14,000 U.S. deaths annually, particularly in immunocompromised and elderly patients who take antibiotics or are under routine medical care.[4] Deaths from *C. difficile* infection increased 400% from 2000 to 2007 and at least 94% of *C. difficile* infections are derived from contact in a medical or patient care facility. Of these, 25% of patients show symptoms in hospitals; the remaining 75% are from patients in nursing homes, treatment clinics and doctor's offices.[4] Since diarrhea is the leading symptom, *C. difficile* is readily spread from one care facility to another and from healthcare worker to patient due to soiled surfaces and equipment. The organism can be difficult to contain due to its ability to produce spores resistant to many commonly used disinfectants and which may lay dormant until transmission occurs. At present, it is estimated that *C. difficile* causes an additional $1 billion in extra healthcare costs annually.[4]

Current methods to detect pathogens like *C. difficile* utilize rapid techniques such as pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR), toxin-typing and mutation analysis. However, surveillance is performed after infection and involves patient testing for positive stool specimens. Thus, an efficient and easy-to-use detection method for monitoring the effectiveness of room disinfection procedures and for identifying environmental reservoirs of *C. difficile* spore contamination prior to the onset of disease could prevent infection, reduce HAI, and potentially reduce the number of deaths due to CDI.[2]

A study by Cadnum et al. compared the use of *Clostridioides difficile* Brucella Broth with Thioglycolic Acid and L-cystine under aerobic conditions versus standard *Clostridioides difficile* Brucella Broth incubated anaerobically for the recovery of *C. difficile* from hospital environmental samples. Their research concluded that broth containing the reducing agents Thioglycolic Acid and L-cystine was significantly more sensitive and specific at recovering *C. difficile* from the environment: 88% and 100%, respectively, versus 51% and 96% for Brucella Broth without these ingredients.[2] In addition, because Brucella Broth supplemented with Thioglycolic Acid and L-cystine can be incubated aerobically and samples obtained using routine environmental monitoring procedures, researchers determined that broth enrichment with these reducing agents was an effective tool at identifying environmental sources of *C. difficile* in healthcare facilities.[2]

Hardy Diagnostics C Diff Banana Broth™ is based on the formulation proposed by Cadnum et al. The basal medium is Brucella Broth supplemented with hemin and vitamin K to promote the growth of fastidious anaerobic microorganisms such as *C. difficile*. L-cystine acts as a growth factor for thiol-dependent or sulfur-containing amino-acid-requiring microorganisms and, in conjunction with the small amount of agar in the formulation, acts as an oxygen reducer to promote the growth of obligately anaerobic bacteria without the need to incubate samples anaerobically.[2,8] In addition, mannitol is used instead of glucose to prevent glucose repression of *C. difficile* cytotoxin synthesis and release.[7] Mannitol fermentation is also an important characteristic of *C. difficile* identification and can be used to differentiate this species from *C. sporogenes*, a similar organism.[6-8] Lysozyme, thioglycolic acid, and sodium taurocholate are included to promote *C. difficile* spore germination from environmental samples, and the pH of the medium is optimized to facilitate fermentation of mannitol by germinating spores of *C. difficile* via the alkaline up-regulated PEP-phosphotransferase (PTS) transport system.[1,2] Cycloserine and cefoxitin are selective agents to inhibit the growth of unwanted environmental organisms. Neutral red is the pH indicator and sodium bicarbonate acts as a buffering agent to maintain alkaline conditions required to promote mannitol fermentation.[10]

Confirmation of *C. difficile* should be performed on positive C Diff Banana Broth™ tubes using established methods. If confirmed, corrective measures using a sporicidal disinfectant can be taken to eliminate environmental reservoirs from facilities and equipment to prevent cross-contamination and infection.[2-4]

FORMULA

Ingredients per liter of deionized water:*

Brucella Broth	28.0gm
D-Mannitol	6.0gm
Sodium Bicarbonate	5.0ml
Neutral Red	5.0ml
Vitamin K1 (1mg/ml)	1.0ml
Hemin	1.0ml
Thioglycolic Acid	1.0gm
L-Cystine	1.0gm
Sodium Taurocholate	0.5gm
D-Cycloserine	500.0mg
Cefoxitin	16.0mg
Lysozyme	5.0mg
Agar	1.0gm

Final pH 7.6 +/- 0.2 at 25 degrees C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Sample Collection: As a general rule, potentially infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Consult laboratory procedures or listed references for information on sample collection and environmental monitoring procedures.[1-9]

Dry sterile swabs (Cat. no. 972029) pre-moistened with a sterile diluent such as Saline, 0.85% (Cat. no. R45)can be used to obtain samples from environmental surfaces. For assessing the efficacy of disinfection procedures, a neutralizing broth such as D/E (Dey-Engley) Neutralizing Broth (Cat. no. K108) should be used instead of physiological saline.

Verify the swab can be broken off into and fit securely in the C Diff Banana Broth™ tube during incubation prior to use. Flocked swabs (Cat. no. 972029) designed to elute the sample may be pre-moistened with a sterile diluent then placed directly into C Diff Banana Broth™ tubes after sampling for best results. In addition, flocked transport swabs such as ESwab™ may also be used for environmental monitoring and swabs placed directly into C Diff Banana Broth™ tubes after sampling. See procedure step 2b for inoculation of C Diff Banana Broth™ from a transport medium.

A swab with a wooden shaft should not be used for sample collection when using molecular confirmatory methods, as this may interfere with PCR results or other molecular assays.

General Procedure for Collecting Environmental Swab Samples:

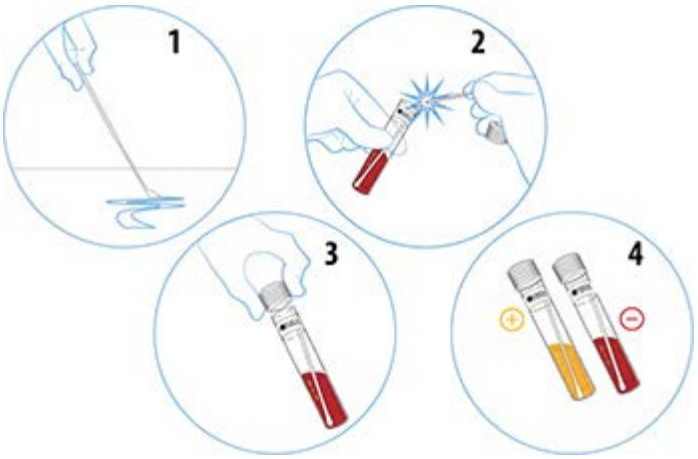
Collect the sample by aseptically rubbing a pre-moistened swab over the sample area (approximately 50cm^2), reversing directions between strokes, and return the swab to the collection device or place the swab directly into C Diff Banana Broth™. When sampling bedding, curved or irregular surfaces, run the swab over the surface of the object as indicated to approximate a similar sampling area. If the sample is not immediately taken to the lab for processing, it can be refrigerated for up to 24 hours prior to analysis. See the **Inoculation Study** details below for delayed incubation.

Method of Use for C Diff Banana Broth™:

1. Allow the medium to warm to room temperature prior to inoculation.

2a. Inoculate the broth by inserting the environmental sample swab directly into the bottom of the tube. Rotate the swab to emulsify the sample in the broth and carefully break the swab shaft, leaving the tip of the swab at the bottom of the tube during incubation. Replace the cap and twist down tightly to seal the tube. It is important that the caps be **tightly sealed** during incubation in order to create an atmosphere of reduced oxygen tension necessary for anaerobic conditions at the bottom of the tube. Refer to the below illustration when sampling. See the **Inoculation Study** details below for delayed incubation.

2b. Alternatively, transport media systems can also be used for surface sampling. Follow the manufacturer's instructions for use (IFU) for storage conditions and holding time prior to inoculation. If the transport medium contains a flocked or elution swab such as ESwab™, collect the sample and place the swab directly into the C Diff Banana Broth™ tube after sampling. Do not return flocked or elution swabs to the transport medium tube, as this may affect the sensitivity of the test. Cap the tube tightly as outlined in 2a. See the **Inoculation Study** details below for delayed incubation.



3. Incubate inoculated tubes with swabs aerobically with tight caps at 35-37 degrees C. for 48 to 72 hours.

4. Observe daily for development of a yellow color in the medium. Any yellow color change is indicative of a positive reaction.

5. Confirmation of *C. difficile* should be performed on positive C Diff Banana Broth™ tubes using established methods, so corrective measures can be taken to eliminate environmental reservoirs from facilities and equipment to prevent cross-contamination and infection.

INTERPRETATION OF RESULTS

Development of turbidity and any yellow color change in the medium after incubation is indicative of growth and a positive mannitol fermentation reaction and suggests the presence of *C. difficile*. Perform confirmatory tests on positive tubes as outlined in the references or as established by laboratory procedure.[4,10]

Lack of any yellow color development is indicative of a negative mannitol fermentation reaction and suggests the absence of *C. difficile*.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The medium is designed to be a presence/absence broth. Therefore, any yellow color development in the medium

during incubation is indicative of a positive result. Further testing is required for complete identification.

A delayed incubation of environmental swabs placed directly into C Diff Banana Broth™ exceeding 8 hours may result in a weak reaction or erroneous results. It is advised that tubes of C Diff Banana Broth™ be incubated within 8 hours of placing the swab into the tube for best results. See the **Inoculation Study** details below for delayed incubation.

Established confirmatory methods such as *C. difficile* latex tests, enzyme immunoassays (EIA) for toxins A and B or glutamate dehydrogenase, spectrophotometric analysis, and PCR tests for toxin B genes are required for confirmatory testing following culture in C Diff Banana Broth™.[(2)]

This product should be incubated with the caps tightly sealed to create an atmosphere of reduced oxygen tension required to support the growth of *C. difficile*. Failure to incubate with tight caps may produce delayed reactions or erroneous results.

Sample testing should take into account the confirmatory assay being performed and the type of swab recommended for use with the assay as indicated by the manufacturer's technical literature or instructions for use (IFU).

Failure to place flocked swabs directly into C Diff Banana Broth™ upon sampling or to incubate environmental sample swabs in C Diff Banana Broth™ may result in erroneous results.

When using a transport medium for sampling with delayed inoculation into C Diff Banana Broth™, follow the manufacturer's instructions for use (IFU) for storage conditions and holding time prior to use. For flocked or elution swab transport systems such as ESwab™, place the swab directly into the C Diff Banana Broth™ tube for best results. Failure to place flocked or elution swabs directly into the C Diff Banana Broth™ tube after sampling may result in reduced sensitivity of the test.

The design and implementation of a microbiological environmental monitoring program should be established prior to initiating sample collection to ensure the program has established a sampling and site plan, detects change, captures trends, and implements appropriate countermeasures.[(9)]

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs (Cat. no. FS1HD), applicator sticks, diluents such as Saline, 0.85% (Cat. no. R45) or D/E Neutralizing Broth (Cat. no. K108), transport swabs, other culture media, confirmatory tests such as *C. difficile* latex test, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Clostridioides difficile ATCC® 9689	B	48-72hr	35°C	Aerobic**	Growth; color change from red to yellow
Clostridium perfringens ATCC® 13124	B	48-72hr	35°C	Aerobic**	Growth; slight turbidity, no color change
Enterococcus faecalis ATCC® 29212	B	48-72hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Tubes incubated with tight caps to create an atmosphere of reduced oxygen tension.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

C Diff Banana Broth™ should appear hazy, and cherry red in color.

An uninoculated tube of C Diff Banana Broth™ compared to a positive tube of *Clostridioides difficile* (ATCC® 9689) grown for 48 hours at 35 degrees C.

INOCULATION STUDY[11]

Because the product is intended for use on environmental samples, delayed incubation may occur between sample collection and delivery of the sample to the laboratory for further processing. Therefore, an in-house inoculation study was performed to replicate placing environmental swabs containing *C. difficile* into C Diff Banana Broth™ with various delayed incubation intervals (4 hours, 8 hours, 18 hours, and 24 hours) to replicate user sampling, and to determine if there was any impact to the performance of C Diff Banana Broth™.

Based on the above in-house study, environmental swabs placed directly into C Diff Banana Broth™ with a delayed incubation period of up to 8 hours showed no measurable effect on the performance of C Diff Banana Broth™.

REFERENCES

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ATCC is a registered trademark of the American Type Culture Collection.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]