QUIDEL | AmpliVue®

Anytime, Anywhere Molecular Assays

C. difficile Assay

Frequently Asked Questions

General

Do you have a proficiency recommendation to validate the AmpliVue C. difficile Assay?
Check with your local and state regulation for proficiency requirements. If you are using the CAP proficiency, Stool Pathogens (SP) is the evaluation. For the API proficiency, 347 or 350 *C. difficile* toxin or antigen is the evaluation we suggest.

What is the CMS suggested CPT code and National Limit amount for the AmpliVue C. difficile Assay?
The suggested CPT code is 87493.* The Medicare National Limit amount** is $47.80. For reimbursement information and support, please visit our website at quidel.com or contact Quidel directly at **800.874.1517 Option 2**, or via e-mail at technicalsupport@quidel.com.

Is the AmpliVue C. difficile Assay FDA cleared?
Yes. It has been FDA cleared. K123355.

I would like to run Limits of Detection. Are your controls quantified?
No. Quidel Molecular Control Sets are unassayed controls. Quantification of controls is the responsibility of the end user.

Are there any special licensing or certifications required to run the AmpliVue C. difficile Assay?
The qualifications for laboratories performing a moderately complex test can be found on the CDC website at **cdc.gov**. For additional information you should contact your State Agency CLIA Contact.

What are the requirements for setting up a correlation study?
The assay should be treated in the same way any other FDA-cleared moderate complexity test brought into the laboratory is treated. There is nothing written in the regulations, that we are aware of, that identifies an assay like AmpliVue requiring anything additional.

Can I use any heat block for the lysis and amplification steps?
Both the 95°C Lysis Block and the 64°C Amplification Block are available from Quidel. The 95°C Lysis Block is designed specifically with holes to fit the buffer tubes and maintains a temperature of 95°C ± 2°C. The catalog number for this heat block is M217 (Domestic) or M218 (International). The 64°C Amplification Block is designed specifically with holes to fit the reaction tubes, maintains a temperature of 64°C ± 2°C and has a heated lid. The catalog number for this block is M215.The heat block that is used for the amplification process must have a heated lid. There are inserts for the heat blocks that can be bought from other suppliers that may fit the tubes, but it is extremely important that the block and insert being used will allow the tubes to remain in contact with the block for optimal and even heat transfer. If using

an alternate heat block, please ensure that the heat block maintains the specified temperature for the entire heating period.

What is the shelf life of the kit?
The kit shelf life is 24 months from the date of manufacture. Actual expiration date of product shipped will vary depending on lot availability.

If my kit was placed in a freezer upon arrival, can I still use it?
It is recommended that the kit not be used. The proper storage condition of the unopened kit is 2°C to 8°C.

Can I purchase the reagents individually?
The reagents are produced in a kit format. Individual reagents are not available for purchase. If a vial is damaged upon receipt, please contact Quidel Technical Support at **800.874.1517 Option 2,** or via e-mail at technicalsupport@quidel.com for replacement arrangements.

What is the concentration of the process control?
That is proprietary information.

Can a sample with another manufacturer's process control be used in your assays?
No. The sample must have a Quidel process control in it; therefore, you should not use a sample lysed using another kit.

Why is the C line not necessary to call a positive result?
The process control is intended to show any inhibition during the assay. A positive result tells you that no inhibition has taken place, the sample has been processed correctly, and the integrity of the reagents and Cassette detection has been confirmed. This occurs because of an overabundance of amplicons which generate competition with the test targets.

What if I can't see the fluid flowing up the detection strip in the Cassette?
If liquid is not flowing in the Cassette then it is recommended that the assay be repeated.

What is the target for the AmpliVue C. difficile Assay?
A conserved fragment of the *C. difficile* DNA, which is intact in all known A+B+ and A−B+ toxinotypes of *C. difficile.* Organisms described as A+B+ or A−B+ are based on the actual detection of the toxins. The AmpliVue C. difficile Assay is based on sequence analysis and the presence of the *tcdA* and *tcdB* genes, not the presence of toxins.

What is the CLIA quality control policy for these tests?
Based on the Memorandum the Office of Clinical Standards and Quality/Survey Certification Group there are two mechanisms for meeting the federal guidelines:

- The default mechanism is to follow 42 CFR 493.1256(d)(3) – which states that two levels of external quality control per day of patient testing will be performed. All manufacturer's instructions must be followed, per 42 CFR 493.1256(d)(2);

- A new program (Individualized Quality Control Plan (IQCP)) has been announced. The Centers for Medicare & Medicaid Services (CMS) is incorporating into the Interpretive Guidelines (IG), based on 42 CFR 493.1250, key concepts and graphics from Clinical and Laboratory Standards Institute (CLSI) Evaluation Protocol-23 (EP-23), 'Laboratory Quality Control Based on Risk Management,' as alternative Clinical Laboratory Improvement Amendment (CLIA) Quality

Control (QC) policy. For more information please request the Quidel technical bulletin for AmpliVue Assay External Quality Control.

What is the CAP quality control requirement for these tests?
CAP Molecular MOL.34220 or MIC.63262: Validation studies must include daily comparisons of external controls to built-in controls for at least 20 consecutive days. Then external controls are run for each new lot number and new shipment and before use or every 30 days whichever is more frequent.

How many tests can I get out of the Quidel Molecular C. difficile Control Set (Cat. #M108)?
Approximately 40 AmpliVue Cassettes can be run from each control set.

What is the approximate test time for the AmpliVue assay?
The total assay time is approximately 80 minutes.

Does the AmpliVue C. difficile Assay detect hypervirulent strains?
The AmpliVue C. difficile Assay has been shown to detect hypervirulent strains, but will not differentiate them from other toxigenic genotypes.

Is it relevant which toxins are recognized for the clinical management of patients?
No. There is no relevance in what toxins are actually produced. As long as it's toxigenic *C. difficile*, then the patient is considered *C. difficile* positive and treated with the same drugs according to standard clinical practices.

Is it important to identify the toxins to determine the bacteria toxigenicity?
No, because the toxins degrade quite rapidly at room temperature and the toxin A or B Enzyme Immunoassays (EIA) have differing sensitivities to different strains of *C. difficile*. The EIA's have a very low sensitivity (approximately 50%), which makes them very unreliable in terms of being able to detect the toxins. Molecular assays (whether they target *tcdA* or *tcdB*) generally detect toxigenic *C. difficile* and do not detect non-toxigenic *C. difficile*. The genes are far more stable than the proteins and they end up being a much more reliable test for the presence of toxigenic *C. difficile* than the EIA's.[1]

Presence of the *C. difficile* bacteria but without toxins, is this important from a clinical point of view?
There is no diagnostic need to determine if someone is an asymptomatic carrier of toxigenic *C. difficile* in stool as this is still considered to be quite rare (2-3% of the population) based on standard clinical practices. Molecular tests are sensitive enough to pick up carriers; however, because they all specify that loose or unformed stool be the specimen tested (rather than solid stool), it is highly unlikely that you would pick up a carrier. If a hospital would like to use an assay to detect carriers, then they could use a molecular test but it would need to be validated for this use.[2]

Where can I find up-to-date news and information on C. difficile?
The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) post information on their websites:
http://www.who.int/
http://cdc.gov/

Frequently Asked Questions

What is the sensitivity/specificity of the AmpliVue C. difficile Assay?
Performance characteristics of the AmpliVue C. difficile Assay were established at four sites across the United States. A description of this trial and the results can be found on page 7 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What is the precision of the AmpliVue C. difficile Assay?
The precision of the AmpliVue C. difficile Assay was demonstrated in a separate study. Data for this study can be found on pages 8-9 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What is the limit of detection (LOD) for C. difficile using this kit?
The analytical limit of detection of the AmpliVue C. difficile Assay can be found on page 7 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What detection technology does the AmpliVue C. difficile Assay use?
The AmpliVue C. difficile Assay utilizes helicase-dependent amplification (HDA) for the amplification of a highly conserved fragment of the Toxin A gene sequence and a self-contained disposable amplification detection device that allows for visual evaluation of assay results. An expanded description of the principle of AmpliVue C. difficile Assay can be found on page 2 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What does the AmpliVue C. difficile Assay kit contain?
The materials provided in each kit are listed on page 3 of AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What sample types can I use with the AmpliVue C. difficile Assay?
How should specimens be handled and stored prior to the heat lysis process?
What container types are acceptable to be used with the assay?
The specimen collection, storage and handling requirements for the AmpliVue C. difficile Assay can be found on page 4 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What are the storage conditions for the kit and the components?
The storage conditions for the AmpliVue C. difficile Assay kit and components can be found on page 3 of the AmpliVue C. difficile Assay kit Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What are Quidel's quality control recommendations for this test?
Quidel's quality control recommendations are provided on page 6 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

Does Quidel provide an external quality control for the AmpliVue C. difficile Assay?
The AmpliVue C. difficile Assay does not include an external control as part of the kit. A suggested external control is listed on page 3 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What do I do if I get a negative result for the Positive Control or an invalid result for the Negative Control?
A full description of External Control use is provided on page 6 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What should I do if my process control line (C line) does not show up?
An interpretation table of results is provided on page 6 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What can cause a false positive with a Negative Control or patient sample?
A list of warnings and precautions is provided on page 3 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

This list covers many of the identified causes of false positive results seen with many molecular test systems.

What can cause a false negative with a Positive control or patient sample?
A list of limitations is provided on page 7 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

This list covers many of the identified causes of false negative results seen with many molecular test systems.

How long are samples stable in Lysis Buffer?
Stabilities are listed in the Note sections in the Assay Procedure. This information can be found on page 4 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What temperature do the samples need to be lysed at?
This information is contained in the Assay Procedure found on page 4 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What if I forgot to heat lyse my samples?
The Assay Procedure is described on pages 4- 5 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

Failure to follow this protocol invalidates the test result.

What temperature do the samples need to be amplified at?
This information is supplied in the Assay Procedure found on page 5 of the AmpliVue C. difficile Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

How long after the 10-minute read time are results valid?
This portion of the Assay Procedure is described on page 5 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

Can I use the results if I forgot to vortex my tubes?
Failure to vortex the tubes is a major deviation from the Package Insert. It is recommended that the assay be repeated. The complete Assay Procedure can be found on pages 4-5 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

What does the T1 line tell me?
The T1 line is not used on this assay. Interpretation of Results can be found on page 6 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

Refer to the Package Insert on our website at **quidel.com** for additional performance claims.

Customer support and technical assistance information is provided on page 12 of the AmpliVue C. difficile Assay Package Insert:
http://www.quidel.com/sites/quidel.com/files/product/documents/AmpliVue%20C%20diff%20PI%2040814.pdf

References:

1. Miyajima F., Roberts P., Swale A., Price V., Jones M., Horan M., et al. Characterisation and carriage ratio of *Clostridium difficile* strains isolated from a community-dwelling elderly population in the United Kingdom. *PLoS One*. 2011;6(8):e22804. doi: 10.1371/journal.pone .0022804. Epub 2011 Aug 23.

2. Niyogi S.K., Dutta D., Bhattacharya M.K., Bhattacharya S.K. Frequency of isolation of toxigenic *Clostridium difficile* from healthy adults. *Indian J. Med. Res*. 1997 Dec;106:497-9.

*This information is being provided as a reference, for informational purposes only, with no expressed or implied warranty and does not purport to provide legal or certified coding advice. ***Under Federal and State law, it is the individual provider's responsibility to determine appropriate coding, charges and claims for a particular service.*** Policies regarding appropriate coding and payment levels can vary greatly from payer to payer and change over time. Quidel Corporation strongly recommends that providers contact their contracted payers to determine appropriate coding and charge or payment levels prior to submitting claims.

**For state by state fee schedule go to www.cms.gov.

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