# CCFA MODIFIED w/ HORSE BLOOD

## INTENDED USE
Remel CCFA Modified w/ Horse Blood is a solid medium recommended for use in qualitative procedures for selective and differential isolation of *Clostridium difficile*.

## SUMMARY AND EXPLANATION
*C. difficile* was first isolated in 1935 and until the 1970s it was believed to be nonpathogenic for humans.[1] In the late 1970s, *C. difficile* was reported to be a major cause of pseudomembranous colitis (antibiotic-associated colitis) or CDAD (*C. difficile*-associated disease).[2,3] In 1979, George et al. recommended the use of modified McClung-Toabe Agar containing cycloserine, cefoxitin, and fructose (CCFA) for isolation of *C. difficile* from clinical specimens.[4] CCFA was further modified by omitting egg yolk (*C. difficile* is lecithinase- and lipase-negative) and horse blood was added to provide extra enrichment. A study by Marler et al. in 1992 demonstrated CCFA Modified w/ Horse Blood produced better growth of *C. difficile* than other media tested.[5]

## PRINCIPLE
Proteose peptone supplies carbon, nitrogen, vitamins, and minerals required for the growth of *C. difficile.* Fructose is a carbon source of energy. Sodium chloride and magnesium chloride are sources of essential electrolytes and maintain osmotic equilibrium. Disodium phosphate and monopotassium phosphate are buffers and also provide phosphates. The antibiotics, cycloserine and cefoxitin, are selective agents. Cycloserine is most active against *Escherichia coli* with other gram-negative bacilli and streptococci slightly inhibited. Cefoxitin is a broad spectrum antibiotic active against a variety of gram-positive and gram-negative bacteria, with the exception of *C. difficile* and *Enterococcus faecalis*. Horse blood provides extra enrichment allowing for better growth of *C. difficile*.

## REAGENTS (CLASSICAL FORMULA)*

| | | | | | |
|---|---|---|---|---|---|
| Proteose Peptone | 40.0 | g | Magnesium Chloride | 0.1 | g |
| Fructose | 6.0 | g | Cycloserine | 250.0 | mg |
| Disodium Phosphate | 5.0 | g | Cefoxitin | 16.0 | mg |
| Sodium Chloride | 2.0 | g | Horse Blood | 7 | % |
| Monopotassium Phosphate | 1.0 | g | Agar | 20.0 | g |
| | | | Demineralized Water | 1000.0 | ml |

pH 7.6 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

## PROCEDURE
1. Prior to use, reduce the plates for a minimum of 24 hours by placing them in an anaerobic environment at room temperature.
2. Inoculate specimens for anaerobic culture on both selective and nonselective media as soon as possible after receipt in the laboratory; streak plates for isolation.
3. Incubate anaerobically for 48-72 hours at 33-37°C.
4. Following incubation, observe the plate for growth of flat, circular colonies with filamentous edges.
5. Confirm anaerobic growth by Gram stain and subculture to a blood agar plate incubated in ambient air
6. Consult appropriate references for additional tests to confirm the presence of *C. difficile*.[6,7]

## QUALITY CONTROL
All lot numbers of CCFA Modified w/ Horse Blood have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

| CONTROL | INCUBATION | RESULTS |
|---|---|---|
| *Clostridium difficile* ATCC® 9689 | Anaerobic, up to 72h @ 33-37°C | Good growth |
| *Bacteroides fragilis* ATCC® 25285 | Anaerobic, up to 48h @ 33-37°C | Inhibition (partial to complete) |
| *Clostridium perfringens* ATCC® 13124 | Anaerobic, up to 48h @ 33-37°C | Inhibition (partial to complete) |
| *Escherichia coli* ATCC® 25922 | Ambient, 18-24h @ 33-37°C | Inhibition (partial to complete) |
| *Staphylococcus aureus* ATCC® 25923 | Ambient, 18-24h @ 33-37°C | Inhibition (partial to complete) |

## LIMITATIONS
1. Other organisms may grow on this medium but they do not demonstrate the characteristic colonies described for *C. difficile*.
2. Typical Gram stain morphology of *C. difficile* may not be evident in colonies selected from this medium because of the antibiotics present. Subculture to anaerobic blood agar to obtain characteristic morphology.[7]

## BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.




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