C. difficile TOXIN A/B

I.   Introduction

C. difficile Tox A/B is an enzyme immunoassay for the detection of toxins A and B produced by toxigenic strains of *C. difficile*. It can be used to detect toxins A and B in fecal specimens from persons suspected of having *C. difficile* disease.

II.   Specimen Collection and Processing

Collect stool specimen in clean Starplex container, and send to the Virology laboratory as soon as possible.
Stool collected in Enteric Transport Medium , or in SAF is not suitable for this assay.

a.   Dispense 600 ul of diluent into 2 ml microtube.
b.   Add 150 ul of liquid stool (mix stool well first) or approx. 6 mm diameter of solid stool.
c.   Vortex for 10 seconds, spin for 5 minutes at # 14 in Eppendorf microcentrifuge. Load onto Behring 2000 Analyser sample rack according to worklist for testing.

III.   Procedure

Behring 2000 Analyzer

i)   Reagent Preparation:

1.   Prepare Behring Wash Solution as needed. e.g.100 ml of Wash Solution +1900 ml of distilled H_2O. Store at 4 ^{0}C. Fill up Wash Solution in Behring 2000 when needed.

2.   Prepare *C. difficle* working buffer: 50 ml of 20X Wash Buffer Concentrate + 950 ml of D H_2O. Store at 4 ^{0}C. Fill up *C. difficile* working buffer in Behring 2000 Analyser when needed.

3. Pour Conjugate into Behring medium -sized container and labeled as 'Conjugate'.
   Pour Substrate into Behring large-sized container and labeled as 'Substrate'.
   Pour Stop Solution into Behring medium-sized container and labeled as 'Stop Solution'.
   All these reagents can be stored in the respective containers in 4^0C refrigerator.
   Use new containers for each new Lot #.

4. Bring microwell pouch and the above mentioned containers to room temperature before use.

5. Empty waste, change D H_2O and clean control bottles / replace with new controls every Friday.

ii)   Assay Method:

1. Turn hard drive and Behring 2000 Analyser 'ON'.

2. Double click on 'Short Cut to BEP 2000'.

3. Enter Technologist's initials, click on 'Don't know Password'.

4. The machine will start 'Initialization' and check all instrument functions. The results of this check are then displayed on the screen.

5. Check DH_2O and Buffer level by opening the bottom part of the Analyzer.

6. Perform 'Washer Assay 'every **morning: see EBV testing, page 21,step 6.**

7. Perform 'Validation Assay' every **Friday: see EBV testing, page 21,step7.**

8. Performing testing:
   a. Remove Reagent Rack #5, and re-load into analyzer.
   b. Remove Reagent Rack # I, and re-load Reagent Rack # I into Analyzer.
   c. Remove Control Rack 'R' and re-load into Analyzer.
   d. Load 1st rack of specimens (1-20) into Analyzer with bar-codes facing the Bar-code reader. Do the same for the subsequent rack(s) (21-40, 41-60).
   e. 'Patient Editor' will appear with the specimen #s. Check that specimen # s are correct .If there is a blank space, click on it and enter the # manually, use 'TAB' to move to another row. Click on the column header, highlight 'C.diff toxin'. Click on the first space and hold , then drag the mouse down the whole column. A '√' (check mark) will appear beside each specimen. Click ' Close' to save. If there is a next rack of specimens, the next page of specimens will appear shortly. Repeat step 'e'.
   f. "Lot specific vales for plate 1', click 'OK', unless a new lot #. If it is a new lot #, just highlight the lot #,type in new lot #. Do the same for expired date.
   g. 'Plate format': will also show the total # of specimens to be tested and # of wells needed. Load *C. difficile* wells onto the plate including controls. Fill in unused space with blanks. Load plate onto plate carrier.
   h. Click 'OK'.
   i. Click on the green→. Initialization will start immediately.
   j. 'LOAD': Purple squares and circles represent Reagents/controls. If you hover the mouse around the squares or circles, it will tell you what it is. Click on the Square, and drag it over to the correct position on the Rack. (Make sure that the arrow is pointed at the center of the square/circle). Chromogen (Substrate) is in the Rack #5, Conjugate & Stop Solution are in Rack # I, and Controls are in Rack # R. If a 'Grey/Green Pipette tip box' appears, it is to load a new set of tips into that space. (Noted the size of tip required: Yellow for 300 µl, Grey for 1100 µl). Load tips. Once finished with loading, click 'OK'.
   k. 'Are you sure that The Resources have been loaded in the correct positions?, Click 'Yes'.
   l. The analyzer will check the levels of the reagents.
   m. 'LOAD PLATE' appears. Under **'Plate ID' enter cd year/month/date (cd030106).**
   n. Load plate carrier into Analyser. Click 'OK'.
   o. 'Are you sure plate layout is correct?', click 'OK'.
   p. 'Downloading Progress' will appear, and then the 'Orange square' will also appear, the testing will start.
   q. To see what stage the test is at: close the inside window on the screen. Click on "+" beside 'Work 2'.
   r. Click on 'Schedule'. You can see the red line move across the screen with each step.

s. When finished, 'Remove plate & Carrier from the system', remove plate and click 'OK'.
t. Click on 'Print Report'.
u. Repeat any specimen that has 'Clot detected' the next working day.
v. Re-capped all reagents, and place the whole kit in the refrigerator.

IV.   Assay validation

Positive Control should read >0.500 at 450/620nm

Negative Control should read < 0.080 at 450/620 nm

V.   Interpretation of Results

Spectrophotometric Dual Wavelength (450/620 nm)

Negative = OD <0.08

Positive = OD >= 0.08

VI.   Reporting

Positive: *Clostridium difficile* toxin detected

Negative: *Clostridium difficile* toxin not detected

If patient is positive for *C. difficile* toxin, phone result to ward, and also phone INFECTION CONTROL NURSE at UHN (TG, TW & PMH), or CHC (in-patient only).

VII.   Quality Control

Negative and positive controls must be included with each run. Refer to a senior technologist if control results are outside of limits or for any other problems with running or reporting the assay.

Run external control (Bartels,lot #G-666 C.difficile Toxin Control) with each new lot. Result filed in External Control Binder. . If result is negative, the run is invalid. Inform Charge/senior technologist, and repeat testing.

CAP provides external proficiency testing.

VIII.　References

1.　　Package insert from TechLab, an ELISA for the detection of *Clostridium difficile* Toxin A and B.
2.　　BEP®2000 Quick Reference Guide, Dade Behring Marburg GMBH.