Illumigene™ *C. difficile* DNA Amplification Test
For Identification of Toxigenic *Clostridium difficile* in Stool Samples

SUMMARY & EXPLANATION OF THE TEST:

The Clinical Microbiology Laboratory offers an FDA-approved molecular diagnostic test (Illumigene™ *C. difficile* DNA amplification test) for the detection of toxigenic *Clostridium difficile* (*C. difficile*) in stool specimens. As a result of the high sensitivity and specificity of the Illumigene™ *C. difficile* DNA amplification assay, it is recommended that only one test (rather than multiple) is necessary within a 7 day period to identify toxigenic *C. difficile* in patients suspected of having *C. difficile*-associated disease (CDAD).

With this assay, a positive test for *C. difficile* indicates the presence of the *C. difficile* toxin A and/or B gene (tcdB) allowing for the presumptive detection of toxigenic *C. difficile.* Results should be interpreted in conjunction with clinical findings. About 2% of normal healthy adults are colonized with *C. difficile*, so a positive test is not definitive for *C. difficile* infection. It also should not be used as a test of cure because dead organism DNA may persist after effective treatment.

The illumigene *C. difficile* assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect a specific *tcdA* region in the pathogenicity locus (PaLoc) which was selected because it is present in all known A+B+ and A-B+ toxin types, and can be found in diarrheal stool samples from patients suspected of having CDAD.

To validate the assay, we tested 266 consecutive patients using the current EIA and the new Illumigene™ assay. Samples with discrepant results were sent for further testing using a different method (PCR). Combined results from the three tests were used as a standard for comparison. According to the standard, the positivity rate in our population was 15.8%. We also found that the EIA and Illumigene™ methods had positivity rates of 7.5% and 16.2% respectively. The Illumigene™ assay had one "false positive" sample, but this patient had previous tests that were positive for *C. difficile.* Compared to the standard, the sensitivity of the EIA and Illumigene™ methods was 47.6% and 100% respectively. Compared to the standard, the specificity of the EIA and Illumigene™ methods was 99.1% and 99.6% respectively. Fifty-nine patients had more than one test during the study period, and 98.3% were classified as positive or negative on the initial Illumigene™ test with no benefit to additional testing. According to the package insert, the Illumigene™ test has a sensitivity, specificity, and correlation of 95.2%, 95.3%, and 95.3% respectively compared a standard of cytotoxic bacterial culture.

Based on these data, it is recommended that in patients suspected of having CDAD, only one test is necessary for establishing whether or not toxigenic *C. difficile* is present in the stool.

The test results must be interpreted in conjunction with clinical findings, and changes in clinical status may indicate the need for further testing. This assay does not distinguish between viable and non-viable organisms, does not identify antimicrobial susceptibility, and does not differentiate the NAP1 (Ribotype 027) strain from other toxigenic strains of *C. difficile*. Colonization at rates up to 50% and higher have been reported in infants, and performance characteristics for specimens from patients less than two years of age have not been established. Colonization rates of up to 32% can be seen in cystic fibrosis patients.

TURN-AROUND-TIME: 1-3 days

SAMPLE REQUIREMENTS:

Unformed stool samples in patients suspected of having *Clostridium difficile*-associated disease (CDAD) can be submitted in Cary-Blair-based media or unpreserved. Unpreserved specimens should be tested as soon as possible.

RESULTS REPORTING:

1) Positive Test: Toxigenic *C. difficile* strain detected
2) Negative Test: No Toxigenic *C. difficile* strain detected

For further questions regarding this assay or other diagnostic assays please contact the clinical microbiology (6646) or molecular diagnostics (5636) laboratories or the director (6444). For further information, please visit our website at: http://www.utoledo.edu/med/depts/path/moldx/index.html