DIAGNOSTIC ASSAYS IN SUPPORT OF PFIZER'S PHASE 3 *C. DIFFICILE* VACCINE EFFICACY STUDY

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Background

Pfizer is currently conducting a Phase 3 study (Clover: CLOstridium difficile Vaccine Efficacy tRial) to evaluate the efficacy of a vaccine composed of *Clostridium difficile* toxoids A and B in adults ≥50 years of age. The primary endpoint for this study is to demonstrate that the vaccine is effective in reducing the incidence of a primary episode of *C. difficile* infection (CDI). To ensure accurate laboratory diagnosis of CDI, Pfizer is using a two-step algorithm for testing stool samples. This algorithm identifies toxigenic *C. difficile* organisms by a nucleic acid amplification test first and positive samples are then evaluated for toxins using Pfizer's novel cell cytotoxicity neutralization assay (CCNA) supported by studies conducted by Planche et al[1] and Polage et al[2] and in diagnostic guidance documents issued by the European Society of Clinical Microbiology[3].

Highlights of the PCR qualification and CCNA validation and clinical validation studies for the PCR and CCNA, respectively, are described. Both assays met all pre-specified acceptance criteria and are suitable for their intended use as diagnostics in *C. difficile* vaccine efficacy and epidemiology studies.

Figure 1: Two-Step Testing Algorithm for Determination of CDI Case Definition

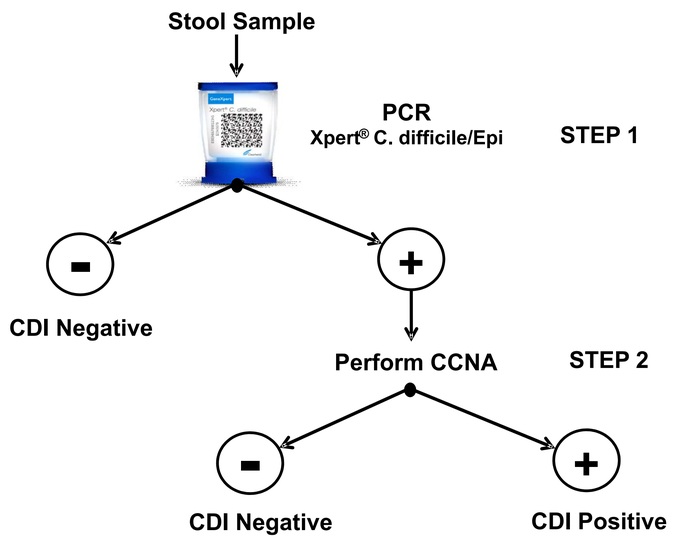
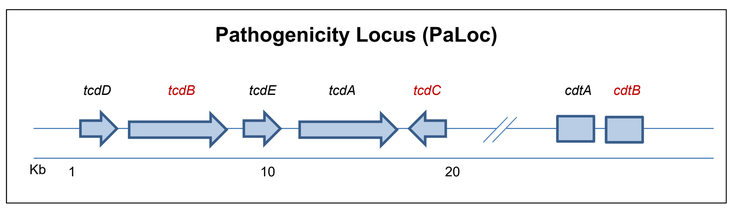


Figure 2: Cepheid's Xpert® *C. difficile*/Epi PCR Assay Detects the Toxin B Gene (*tcdB*), the Binary Toxin Gene (*cdtB*), and the *tcdC* Gene Deletion



Method Qualification of the Cepheid's Xpert® *C. difficile*/Epi PCR Assay

The Cepheid Xpert® *C. difficile*/Epi PCR Assay was qualified at the Pfizer testing facility. The qualification evaluated specificity, sensitivity, precision and accuracy.

I. **Specificity:** Evaluated 50 non toxigenic and non *C. difficile* strains.
Result: No cross-reactivity was observed giving an analytical specificity of 100%.

II. **Sensitivity:** Determined limit of detection (LOD) of 10 *C. difficile* strains spiked in diarrheal stool samples.
Result: LOD for the 10 strains ranged from 344 to 2175 colony forming units (CFU).

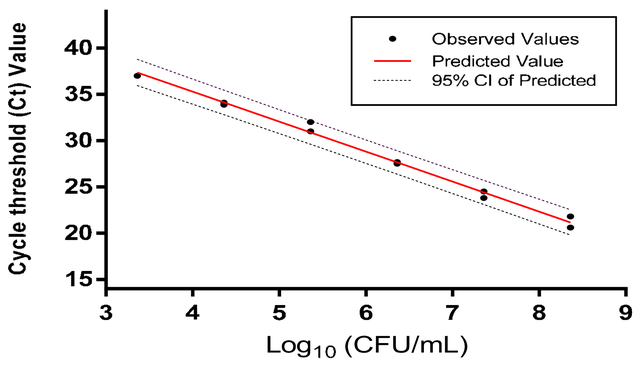
III. **Precision:** Evaluated spiked diarrheal stool samples tested by 2 analysts, using 3 lots of test reagent over 10 test days.
Result: Precision with total Ct RSDs between 0.943 and 1.481 (Table 1) was observed.

V. **Accuracy:** Evaluated the agreement between the expected and observed Ct values and the reference value as predicted by the slope = -3.3 Ct.
Result: Accuracy over 5 logs (Figure 3).

Table 1: Excellent Precision Observed with Cepheid's Xpert® *C. difficile*/Epi PCR Assay

C. difficile Strain	Ct Mean	Relative Standard Deviation (Ct RSD)				
		Cepheid Lot	Analyst	Day	Residual	Total Ct RSD
ATCC-43255	35.8	0.196	0.000	0.526	0.758	**0.943**
PFECD0015	34.5	0.361	0.000	1.244	0.719	**1.481**

Figure 3: Excellent Accuracy Observed over a range of 5 logs



Pfizer's CCNA

Validation: The goal of CCNA is the detection of *C. difficile* toxins in stool samples suspected of CDI. Pfizer's CCNA (Fig. 4) was developed to be sensitive, robust, and reproducible using an objective assay read-out. In contrast, traditional CCNAs rely on the subjective interpretation of an analyst to recognize a cytopathic effect (visual microscopic examination of cell rounding), which is less precise at lower toxin levels. Clinical stool samples are tested in duplicate and scored as positive or negative based on a positivity cutoff. Assay validation [precision (Fig. 5), linearity, accuracy and specificity] confirmed that the assay is suitable for its intended purpose.

Figure 4: Schematic Representation of Pfizer's CCNA

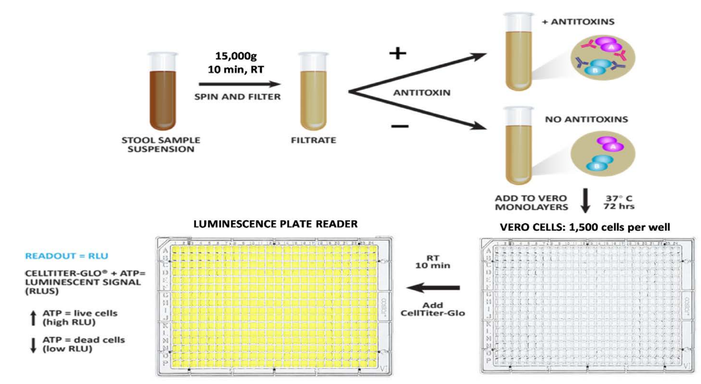
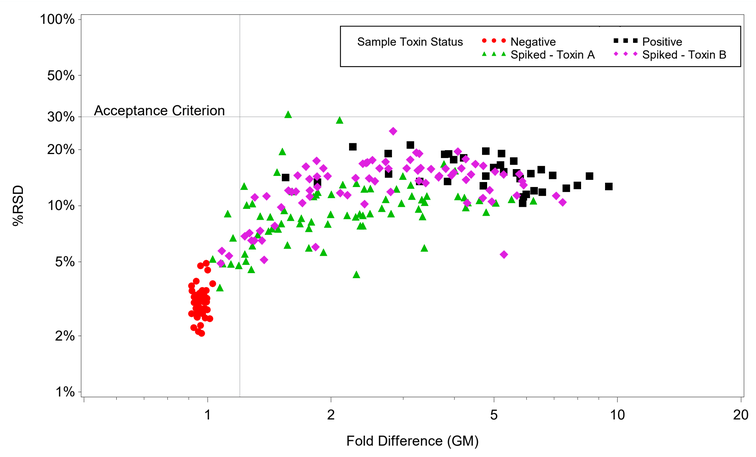


Figure 5: Excellent Precision Observed During Assay Validation



Clinical Validation: Clinical validation of Pfizer's validated CCNA and associated positivity cutoff was analyzed by comparing CCNA test results of suspected CDI samples at both at an accredited reference laboratory (Leeds Teaching Hospitals NHS Trust, Leeds, UK) and Pfizer. Samples were classified by the reference laboratory as either "true negative" [i.e. PCR-/toxin-, (Table 3)], "true positive" [i.e. PCR+/toxin+, (Table 4)] or "potential excretors" [i.e. PCR+/toxin-, (Table 5)]. All samples were confirmed, positive or negative, for the presence of *C. difficile* via Cepheid's Xpert® *C. difficile*/Epi PCR Assay performed at Pfizer (Fig. 6).

Figure 6: Cepheid's Xpert® *C. difficile*/Epi PCR Assay Correctly Confirmed all 93 "True Positives" during Clinical Validation

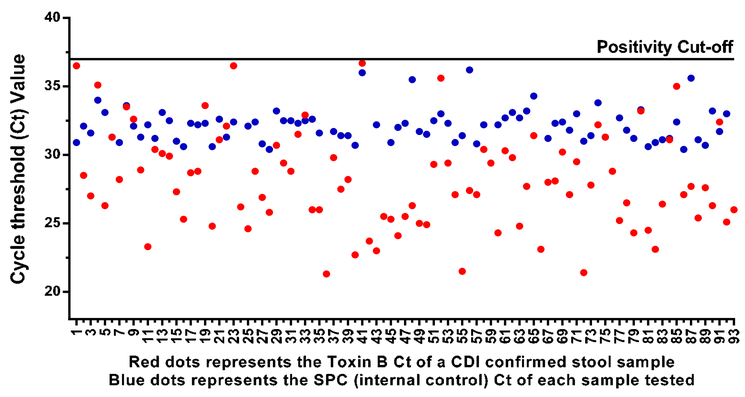


Table 3: 100% Specificity Observed when Testing "True Negatives"

Pfizer's CCNA	Reference Laboratory's CCNA	
	Negative	Positive
Negative	206	0
Positive	0	0

Table 4: 95.7% Sensitivity Observed when Testing "True Positives"

Pfizer's CCNA	Reference Laboratory's CCNA	
	Negative	Positive
Negative	0	4
Positive	0	89

Table 5: 43.5% of Samples Classified as "Potential Excretors" by the Reference Laboratory were Positive in Pfizer's CCNA

Pfizer's CCNA	Reference Laboratory's CCNA	
	Negative	Positive
Negative	13	0
Positive	10	0

Table 6: Pfizer's CCNA is 100X more sensitive than the Traditional CCNA

Toxin B Conc. in Sample (pg/mL)	Pfizer's CCNA	LTHT Result
0	Negative	Negative
5	Positive	Negative
10	Positive	Negative
25	Positive	Negative
50	Positive	Negative
100	Positive	Negative
250	Positive	Negative
500	Positive	Positive
1,000	Positive	Positive
5,000	Positive	Positive
10,000	Positive	Positive

Discussion/Conclusions

- Pfizer uses a 2-step algorithm for laboratory confirmation of CDI based on the detection of the *C . difficile* organism followed by the detection of toxins.
- Pfizer has successfully qualified and validated both diagnostic assays required for clinical testing.
- During clinical validation, both diagnostic assays were shown to be specific and sensitive in the detection of presumed CDI cases.
- Pfizer's CCNA was also shown to be 100X more sensitive than the traditional CCNA.

References

1. Planche TD, Davies KA, Coen PG, et al Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnostic validation study of *C difficile* infection. Lancet Infect Dis 2013; 13:936-45.
2. Polage, CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of *Clostridium difficile* infection in the Molecular Test Era. JAMA Intern Med 2015; 175(11):1792-801.
3. Crobach MJT, Planche T, Eckert C, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clinical Microbiology and Infection 2016; 22:S63-S81.

Acknowledgments

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