Unique Protocol ID: NOWDx COVID-19 Antibody POC

Brief Title: NOWDx Test for the Detection of Antibodies to COVID-19

NCT Number: NCT04690413

Document Date: September 01, 2020

Statistical Analysis Plan

Project Name

NOWDx COVID-19 EUA Blood Test CLIA Waiver Trial; IRB # Pro00045452

Project Personnel

Sponsor Principal Investigator: Beth Cobb
Person(s) performing statistical analyses: Beth Cobb, Shincy Koodathil, Kelsi Thurman, Jessica Thomas, Lexie Hopper

Study Design

Cohort #	Cohort Name	Cohort Size	Prospective or Retrospective?	Case or Control?	Inclusion Criteria	Exclusion Criteria	Data to be collected	Sub-cohorts?
1	Persons who have tested positive for COVID-19 with an emergency use authorized molecular (PCR) test and can furnish said test report; ≥ 7 days post emergency use authorized molecular (PCR) test	30	Prospective	Case	Persons who have tested positive for COVID-19 with an emergency use authorized molecular (PCR) test and can furnish said test report; ≥ 7 days post	Persons <18 years old; persons with limited or no reading skills; persons who previously participated in a NOWDx study; < 7 days post emergency use authorized molecular (PCR) test	Phone Screening Script Responses; Screening/Enrolment Log; ICFs; Surveys; CRFs; Study Visit Checklist and Incentive Receipt forms; EUA molecular (PCR) test report; AE/SAE forms	N/A

					emergency use authorized molecular (PCR) test			
2	Persons who have tested negative for COVID-19 with an emergency use authorized molecular (PCR) test and can furnish said test report; 0-6 days post EUA molecular (PCR) test	30	Prospective	Case	Persons who have tested positive for COVID-19 with an emergency use authorized molecular (PCR) test and can furnish said test report; 0-6 days post emergency use authorized molecular (PCR) test	Persons <18 years old; persons with limited or no reading skills; persons who previously participated in a NOWDx study; > 6 days post emergency use authorized molecular (PCR) test	Phone Screening Script Responses; Screening/Enrolment Log; ICFs; Surveys; CRFs; Study Visit Checklist and Incentive Receipt forms; EUA molecular (PCR) test report; AE/SAE forms	N/A

Samples to be Collected *(Note: must include the 'complete spectrum of patient characteristics' – see FDA Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests)*

Cohort #	Cohort Name	Type(s) of Specimens	Tube type(s)	Quantity	Collection Procedure	Storage Procedure	Handling Procedure
1	Persons who have tested positive for COVID-19 with an emergency use authorized molecular (PCR) test and can furnish said test report; ≥ 7 days post emergency use authorized molecular (PCR) test	Blood; plasma	EDTA	1 EDTA	Standard phlebotomy; fingerstick	≤ -20°C	Study sites will ship plasma from EDTA tubes to NOWDx

2	Persons who have tested negative for COVID-19 with an emergency use authorized molecular (PCR) test and can furnish said test report; 0-6 days post EUA molecular (PCR) test; 0-6 days post emergency use authorized molecular (PCR) test	Blood; plasma	EDTA	1 EDTA	Standard phlebotomy; fingerstick	≤ -20°C	Study sites will ship plasma from EDTA tubes to NOWDx

Comparator Methods

Reference Standard Method: N/A
Non-Reference Standard/Comparative Method: N/A

Power/sample size calculations:

Sample size is determined by FDA EUA Guidance for serology assays.

Intended Use Sites *(minimum = 3)*

Study Site	Address	Site Operators	Recruitment, Specimen Collection, or Both?

Clinical Research Solutions, LLC	2075 Pleasant Plains Extension, Jackson, TN 38305 Ph. 731-984-8400	PI: Melanie Hoppers, M.D. Site operators 1& 2: Aubrey Walgren; William Edwards; Hannah Wells	Both
Comprehensive Clinical Research, LLC	603 Village Blvd. Suite 301. West Palm Beach, FL 33409 (561) 478-3177; (561) 683-1331 (24 hours)	PI: Ronald Ackerman, MD, FACOG Site operators 1& 2: Diana Mann; Tomeko Heard	Both
Del Sol Research Management, LLC	5700 East Pima Street, Suite A. Tucson, AZ 85712 Ph. 520-257-3881	PI: Carl F. Diener, MD Site operators 1 & 2: Antonia Garcia; Krissa Goodin	Both

NOWDx Test/Device – ADEXUSDx® COVID-19 Test

Intended Use	**Intended Operator Experience**	**Test Setting**	**Controls Applied?**	**Specimen Acceptance Criteria**
The ADEXUSDx® COVID-19 Test is an *in vitro* lateral flow immunoassay intended for qualitative detection of total antibodies to SARS-CoV-2 in human whole blood(venous and fingerstick). The ADEXUSDx® COVID-19 Test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-	CLIA Waived operator	Point of Care	Internal and External Controls (applied once per shipment	Capillary blood/ venous whole blood volume sufficient to fill the Fill Zone and reach the Fill Line

CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Testing of human venous whole blood (EDTA) and fingerstick specimens can be conducted in patient care settings authorized to perform CLIA waived tests.			for each lot of tests)	

Reference Standard Test/Device

Intended Use	Intended Operator Experience	Test Setting	Controls Applied?	Specimen Acceptance Criteria
N/A	N/A	N/A	N/A	N/A

Non-Reference Standard/Comparative Method Test/Device – N/A

Intended Use	Intended Operator Experience	Test Setting	Controls Applied?	Specimen Acceptance Criteria
N/A	N/A	N/A	N/A	N/A

FLEX Studies *(to identify potential device deficiencies, including failures and determine the robustness of the test system; See 6-8, CLIA Waiver Guidance 2020*

Operator error FLEX studies	Specimen volume, reading time; unique test characteristics
Specimen integrity and handling FLEX studies	N/A
Reagent Integrity/Visibility FLEX studies	N/A

Hardware, software and electronics integrity FLEX studies	N/A
Stability of calibration and internal controls FLEX studies	N/A
Environmental factors FLEX studies	Temperature and Humidity; Light
Other	N/A

Cross-Reactivity

Not required due to performance of the test in the internal validation study; submitted to FDA on 05/29/2020

Interference

N/A

Method Comparison Studies *(Min: 3 sites; 2 operators; equal distribution b/w operators (min: 10 pos, 10 neg each operator); 30 positive samples, 30 negative samples); PERFORM site specific and overall analyses; also PERFORM for fingerstick and venous sample results separately in comparison to comparator, not to each other)*

The results from this study should be presented as sensitivity and specificity as compared to the comparator method and should have a PPA of 90% and NPA of 95%; 2x2 table format; data to be stratified by days since PCR result; data to be presented separately for each matrix testing method (fingerstick and venous whole blood).

Determining Device Performance with Analyte Concentrations Near the Cutoff *(Dilution studies (see 2020 CLIA Waiver guidance p.29); Goal: find concentrations where the indicator (colored line) is observed 95% (weak positive) or 5% (weak negative) of the time by professional operators); include samples of all concentrations; 60 weak positive aliquots and 60 weak negative aliquots evenly distributed across 3 sites; MASKED & order randomized TO professional OPERATORS)*

N/A

Reproducibility Studies

N/A

Interim Analyses *(See CR-WI-01.21: Clinical Research: Statistical Analysis Plan)*

A bi-weekly interim analysis will be performed, and a presentation will be sent to the CEO of an overview of study recruitment to date. At the discretion of the Director of Operations, said presentations may also include study recruitment per cohort per site, sensitivity & specificity data, and any updates regarding preparation for EUA submission.
Purpose of interim analyses: to monitor completion rate of studies and test performance
Decisions to be made as a result of interim analyses: increase study sites, interim study visit monitoring, retraining, etc.

Derived variables

N/A

Software

We will use Microsoft Office 365 Excel (Version 16.0 or latest version) to perform statistical analyses of data.

Data Summarization

Demographic and clinical characteristics of cohorts will be summarized (in text or table form). Demographic data to be summarized may include gender diversity, ethnicity and racial diversity, age diversity, and education diversity.

Ethnicity and Racial Diversity (RECORD FOR EACH STUDY SITE & COLLECTIVELY):

	Ethnic Categories									
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			Total
Racial Categories	Female	Male	Unknown /Not Reported	Female	Male	Unknown /Not Reported	Female	Male	Unknown /Not Reported	
White										

Black or African American										
American Indian/Alaska Native										
Asian										
Native Hawaiian or Other Pacific Islander										
Unknown										
Total										

Age Diversity (RECORD FOR EACH STUDY SITE & COLLECTIVELY):

Age Range (in years)	# of Males	# of Females
18-24		
25-34		
35-44		
45-54		
55-64		

65+		

Education Diversity (RECORD FOR EACH STUDY SITE & COLLECTIVELY):

Highest degree/level of school completed	# of Males	# of Females
No schooling completed		
Some grade school, no diploma		
High school graduate, diploma, or the equivalent (for example: GED)		
Some college credit, no degree		
Trade/technical/vocational training		
Associate degree		
Bachelor's degree		
Master's degree		
Doctorate degree		

		Comparator Test	
		Positive	Negative
NOWDx COVID-19 Test	Positive	A	D
	Negative	C	B

Sensitivity (AKA the true positive rate): A/(A+C)
Specificity (AKA the true negative rate): B/(B+D)

		Candidate Device Results		
Days since EUA PCR Test	**Number of Samples Tested**	**Total Antibody Positive Results**	**Total Antibody PPA**	**95% CI**
0-6 days	**A**	**a**	**a/A (%)**	
7-13 days	**B**	**b**	**b/B (%)**	
14-20 days	**C**	**c**	**c/C (%)**	
20+ days	**D**	**d**	**d/D(%)**	

SAP Amendment Process *(See CR-WI-01.21: Clinical Research: Statistical Analysis Plan)*

The SAP and any subsequent revisions must be version controlled and recorded in the SAP Revision History Table. The SAP Revision History Table records the following information:

- Author of change
- Version number
- Version date
- Section/content changed

The final SAP version must be approved by all named approvers.

SAP Training

All project personnel will review the SAP and all associated procedures or project documents in detail prior to performance of any project work. Personnel should pose questions to PI to clarify any misunderstandings or areas of confusion. Training should be documented using the Statistical Analysis Plan Training Log CR-LOG-01.25.

Author of this Statistical Analysis Plan (Name) and, if different to that of the Sponsor Principal Investigator, their **telephone & email contact details**
Shincy Koodathil; 479-966-4514; Shincy.koodathil@nowdx.com

Approved by:

Sponsor Principal Investigator (PI)

Beth L Cobb 09/01/2020

Name Date

Director of Operations (if different than Sponsor PI)

Name Date