EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
GeneSTAT.MDx *Coccidioides* Assay
DECISION SUMMARY

A. DEN Number:

DEN170041

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the GeneSTAT.MDx *Coccidioides* Assay

C. Measurands:

DNA sequences of *Coccidioides immitis* and *Coccidioides posadasii*

D. Type of Test:

Real-time polymerase chain reaction (PCR) assay

E. Applicant:

DxNA, LLC

F. Proprietary and Established Names:

GeneSTAT.MDx *Coccidioides* Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3376

2. Classification:

   Class II (Special Controls)

3. Product code(s):

   QAA

4. Panel:

   83- Microbiology

H. Indications for Use:

1. Indications for use:

   The GeneSTAT.MDx *Coccidioides* Assay is a qualitative real-time polymerase chain reaction (PCR) in vitro diagnostic test for the detection of nucleic acids from *Coccidioides immitis* and *Coccidioides posadasii*. The assay does not differentiate between these two species. The assay is performed on bronchial alveolar lavage (BAL) or bronchial wash (BW) specimens from patients presenting with respiratory signs and symptoms consistent with coccidioidomycosis. The GeneSTAT.MDx *Coccidioides* Assay is intended to be used along with clinical findings and other laboratory results as an aid in the diagnosis of coccidioidomycosis in patients with possible or probable exposure to *Coccidioides immitis* or *Coccidioides posadasii*.

2. Special conditions for use statement(s):

   For in vitro diagnostic use only
   For prescription use only

3. Special instrument requirements:

   GeneSTAT System

I. Device Description:

The GeneSTAT.MDx *Coccidioides* Assay is a qualitative real-time PCR-based assay to detect target *Coccidioides spp.* DNA from bronchial alveolar lavage (BAL) or bronchial wash (BW) samples. The sample is prepared by a lysis and extraction process, with the extracted DNA then tested with the GeneSTAT.MDx *Coccidioides* assay on the GeneSTAT System.

The GeneSTAT System is a real-time PCR nucleic acid testing platform. The GeneSTAT system consists of:

- The single-use, disposable, assay-specific Test Module cartridge that contains the real-time PCR reagents, internal control, reaction wells, and test parameters. The single-use cartridge contains all the necessary reagents for amplification and detection of the *Coccidioides* target DNA as well as a human DNA control sequence that is used to monitor the presence of inhibitors in the PCR reaction and to ensure that the sample preparation process is adequate. All information to run the assay as well as lot number and expiration date is contained on the Radio-Frequency Identification (RFID) tag on the cartridge. The cartridge contains two PCR reaction wells. One well contains PCR reagents for amplification and detection of *Coccidioides* DNA and the human gene internal control (Reaction Well 1). The second well (Reaction Well 2) contains the same reagents for amplification/detection of *Coccidioides* DNA as used in Reaction Well 1 but does not contain the PCR reagents to detect the internal control.

- The GeneSTAT Analyzer instrument that performs the mechanical, optical, and fluidic actions upon the Test Module to perform the PCR reactions. The instrument generates, analyzes, and transmits results to a connected pre-configured computer for presentation to the user. One single-use Test Module can be tested at a time in each GeneSTAT Analyzer. Up to four GeneSTAT Analyzers can be connected to single computer.
- A MS Windows based laptop computer
- The associated software and firmware required to run the test, analyze the results, and present the results to the user.
- The Performance Verification Test Cartridges (PVT) that are used to periodically verify that the analyzer is functioning within acceptable limits. A successful PVT run is required to activate the analyzer. The PVT should be run at minimum every six months, and it is recommended that a PVT should be run monthly.

Materials provided:

1. Lyticase
2. GeneSTAT Sample Vials
3. GeneSTAT.MDx *Coccidioides Assay* Test Module Cartridges

Materials required but not provided:

1. GeneSTAT Analyzer instrument
2. Preconfigured computer running Windows 8.1 or greater
3. Performance Verification Test (PVT) Sets

Materials required and available from other suppliers:

1. Microcentrifuge capable of reaching RCF = 20,000 x *g*
2. Centrifuge with rotor(s) capable of holding 15 mL or 50 mL capped centrifuge tubes
3. QIAGEN QIAamp DSP DNA Mini Kit
4. Biosafety Level 2 cabinet
5. SPUTOLYSIN Reagent

J. Standard/Guidance Document Referenced (if applicable):

Not applicable

K. Test Principle:

The GeneSTAT.MDx *Coccidioides* assay is a qualitative real-time PCR-based assay that detects but does not differentiate between DNA sequences of *Coccidioides immitis* and *Coccidioides posadasii,* the causative agents of Valley Fever. The assay detects target DNA that has been previously extracted from bronchial alveolar lavage (BAL) or bronchial wash (BW) samples. The BAL/BW sample preparation process includes the lysis of *Coccidioides* organisms within the sample with sputolysin, and the subsequent extraction and purification of DNA using the QIAGEN QIAamp DSP DNA Mini Kit.

The extracted DNA is placed inside the sample vial, which is then attached to the *Coccidioides* assay cartridge. The cartridge is placed in the GeneSTAT instrument, the RFID tag is read by the instrument, and the user follows a series of data-entry steps, including adding sample ID information, and the run is started. All subsequent steps of the assay process are performed by the GeneSTAT instrument without user intervention, including transfer of sample from the sample vial to the reaction wells in the cartridge, hydration of PCR reagents, PCR amplification and real-time detection of target sequences, and results analysis. The GeneSTAT assay process, from loading the extracted sample into the cartridge to assay result, takes approximately 1.5 hours.

L. Performance Characteristics:

1. Analytical performance:

a. Reproducibility

Between-laboratory reproducibility of the GeneSTAT.MDx *Coccidioides* assay was assessed at three test sites (two external, one internal). Each of the three sites tested three replicates of blinded and randomized samples contrived in negative BAL sample matrix and spiked with attenuated *C. posadasii* spherules (strain Δcts2/Δard1/Δcts3) at three levels of reactivity, expressed in genome equivalents per milliliter (GEq/mL):

(1) Moderate Positive: 3x LoD (20.91 GEq/mL)
(2) Low Positive: 1x LoD (6.97 GEq/mL)
(3) Negative: *Coccidioides*-Negative BAL

Two operators at each site conducted the study over five non-consecutive days, resulting in 30 replicates at each site and 90 replicates overall for each sample reactivity level. Each operator used two GeneSTAT instruments, for a total of 12 instruments overall. Each site tested a different lot of Test Module cartridges. External positive and negative controls were run each day of testing. One Moderate Positive sample and two Negative samples produced Invalid results and were not repeated. Qualitative results are summarized in Table 1, and average Ct and %CV values for the positive samples are summarized in Table 2 for individual sites and across all sites. Qualitative and quantitative analyses conducted by day and by operator are summarized in Table 3, Table 4, Table 5, and Table 6. The reproducibility results are acceptable.

Table 1. Between-Site Reproducibility - Qualitative Analysis

Sample ID	Site 1	Site 2	Site 3	All 3 Sites
	% Agreement	% Agreement	% Agreement	% Agreement [95% CI]
Moderate Positive	96.7% (29/30) †	100% (30/30)	100% (30/30)	98.9% (89/90) [94.0% - 99.8%]
Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) [95.9% - 100%]
Negative BAL	96.7% (29/30) †	100% (30/30)	96.7% (29/30) †	97.8% (88/90) [92.3% - 99.4%]

† One Moderate Positive sample and two Negative samples produced Invalid results and were not repeated

Table 2. Between-Site Reproducibility - Quantitative Analysis

Sample ID	Site 1		Site 2		Site 3		All 3 Sites	
	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)
Moderate Positive	36.8 (6.7%)	33.8 (3.2%)	34.3 (3.7%)	32.9 (2.2%)	36.3 (8.7%)	33.5 (4.3%)	35.8 (6.4%)	33.4 (3.2%)
Low Positive	39.1 (9.3%)	35.3 (4.4%)	37.8 (7.1%)	34.8 (3.6%)	38.0 (9.7%)	35.6 (6.4%)	38.3 (8.7%)	35.3 (4.8%)

Table 3. Reproducibility by Day - Qualitative Analysis

Sample ID	Day 1	Day 2	Day 3	Day 4	Day 5
	% Agreement	% Agreement	% Agreement	% Agreement	% Agreement
Moderate Positive	(b) (4)				
Low Positive					
Negative BAL					

Table 4. Reproducibility by Day - Quantitative Analysis

Sample ID	Day 1		Day 2		Day 3		Day 4		Day 5	
	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)
Mod. Positive	(b) (4)									
Low Positive										

Table 5. Reproducibility by Operator - Qualitative Analysis

Sample ID	Site 1	Site 2	Site 3
	(b) (4)		
Moderate Positive			
Low Positive			
Negative BAL			

Table 6. Reproducibility by Operator - Quantitative Analysis

Sample ID	Site 1				Site 2				Site 3			
	Operator 1		Operator 2		Operator 1		Operator 2		Operator 1		Operator 2	
	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)
Mod. Positive	(b) (4)											
Low Positive												

Within-laboratory Repeatability

Within-laboratory repeatability testing was performed at one internal testing site by two operators using the same panel of blinded challenge samples described for the between-laboratory reproducibility study. The study was conducted over 12 non-consecutive days. Each operator tested two replicates of each panel member per day, resulting in 24 replicates per operator and 48 replicates overall. Qualitative results are summarized in Table 7, and average Ct values and %CV values are shown in Table 8. The results are acceptable.

Table 7. Within-Site Repeatability - Qualitative Analysis

Sample ID	Operator 1 % Agreement [95% CI]	Operator 2 % Agreement [95% CI]	Combined % Agreement [95% CI]
Moderate Positive	100% (24/24) [86.2% - 100%]	100% (24/24) [86.2% - 100%]	100% (48/48) [92.6% - 100%]
Low Positive	87.5% (21/24)† [69.0% - 95.7%]	100% (24/24) [86.2% - 100%]	93.8% (45/48)† [83.2% - 97.9%]
Negative BAL	95.8% (23/24)* [79.8% - 99.3%]	100% (24/24) [86.2% - 100%]	97.9% (47/48)* [89.1% - 99.6%]

†Three Low Positive samples produced Not Detected results and were not repeated.

*One Negative sample produced an Invalid result and was not repeated.

Table 8. Within-Site Repeatability - Quantitative Analysis

Sample ID	Operator 1		Operator 2		Combined	
	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)	Well 1 Avg. Ct (%CV)	Well 2 Avg. Ct (%CV)
Moderate Positive	34.3 (5.4%)	33.2 (4.2%)	34.9 (5.3%)	33.4 (3.2%)	34.6 (5.4%)	33.3 (3.7%)
Low Positive	36.7 (6.8%)	35.5 (4.6%)	37.0 (4.5%)	36.0 (6.2%)	36.9 (5.5%)	35.8 (5.5%)

b. *Linearity/assay Reportable Range:*

Not Applicable

c. *Traceability, Stability, Expected Values (controls, calibrators, or methods):*

Internal Control:

One of the reaction wells in the test cartridge contains two PCR primer/probe sets: a set for the detection of *Coccidioides* spp. DNA and an internal control set to detect human DNA (RNase P gene) that is present in the clinical sample and co-extracted with the *Coccidioides* organism. The internal control is used to verify that the sample processing and DNA purification process occurred efficiently, that the assay reagents and equipment are functioning correctly, and to monitor for the presence of PCR inhibitors in the sample.

External Controls:

External negative and positive quality control samples should be run periodically, and in conformance with local, state and/or federal regulations or requirements. Negative and positive controls should be tested using the same procedure used for patient samples, starting from the first step of the sample extraction process.

Previously characterized BAL/BW samples that have tested negative in the GeneSTAT.MDx *Coccidioides* Assay or commercially available human DNA dissolved in molecular biology grade water or sterile saline should be used as the external negative control. Note: Human DNA must be present in the negative control to avoid generation of an invalid result in the GeneSTAT.MDx *Coccidioides* Assay.

Previously characterized BAL/BW samples that have tested positive in the GeneSTAT.MDx *Coccidioides* Assay or commercially available *Coccidioides* materials suitable for external positive controls (e.g., BEI Resources, Cat. No. NR-166).

Positive and negative controls were run each day of testing during the analytical and clinical testing. Of the total 94 external controls that were run across all sites in the clinical study, one sample produced an initial invalid result (1.1%) which resolved to the expected valid result upon re-test. One positive control out of 47 (2.1%) produced an initial false negative result which resolved to the expected positive result upon re-

test.

d. *Detection Limit:*

The Limit of Detection (LoD) for the GeneSTAT.MDx assay was determined using a stock of attenuated *C. posadasii* spherules (strain Δcts2/Δard1/Δcts3) quantified as spherules per milliliter and genome equivalents per milliliter (GEq/mL). The stock sample was serially diluted in *Coccidioides*-Negative BAL matrix. The LoD of the assay was estimated by testing 20 replicates of five concentrations of *C. posadasii* spherules in BAL matrix by two operators on four GeneSTAT instruments over five days with two assay kit lots. The lowest concentration of *C. posadasii* spherules that gave a minimum 95% detection rate was then retested with an additional 60 replicates at that dilution to confirm the LoD. The LoD of the GeneSTAT.MDx *Coccidioides* assay performed on the GeneSTAT System is 6.97 GEq/mL. An attenuated strain of *C. immitis* was not available for testing. The same testing scheme was also used to determine the LoD of pre-extracted *C. posadasii* and *C. immitis* DNA diluted in a synthetic BAL matrix composed of 0.9% w/v sterile saline and 0.3 µg/mL human genomic DNA in 0.5% w/v carboxymethyl cellulose. The confirmed LoD for both *C. posadasii* DNA and *C. immitis* DNA was 10.0 GEq/mL.

e. *Analytical Reactivity (Inclusivity):*

The inclusivity of the GeneSTAT.MDx *Coccidioides* assay was assessed by testing previously extracted DNA from different *C. posadasii* and *C. immitis* isolates representing temporal and geographical diversity of the two *Coccidioides* strains in the US (Table 9). Twenty replicates of five different DNA samples from both strains were tested at a 3x LoD concentration (30.0 GEq/mL).

All 20 replicate samples of the five clinical isolates of *C. posadasii* DNA were detected in the assay. Likewise, all 20 replicate samples of the five clinical isolates of *C. immitis* DNA were detected in the assay. An invalid result was observed for one strain replicate; however, the expected positive result was produced when the sample was re-tested per the assay instructions. The results are acceptable.

Table 9. Inclusivity Panel

Strain	Collection Site
C. posadasii	Yuma, AZ
C. posadasii	San Joaquin, CA
C. posadasii	Maricopa County, AZ
C. posadasii	Tucson, AZ
C. posadasii	San Antonio, TX
C. immitis	Monterey, CA
C. immitis	Barstow, CA
C. immitis	San Joaquin, CA
C. immitis	San Joaquin, CA
C. immitis	Tucson, AZ

f. Analytical Specificity:

Cross reactivity/ Microbial Interference

The GeneSTAT.MDx *Coccidioides* assay was used to test a panel of 47 non-target viruses, bacteria, fungi, and DNA preparations that may be present in respiratory specimens (Table 10). For cross-reactivity testing, each organism was spiked into negative BAL/BW matrix at $\geq 10^6$ CFU/mL for bacteria and fungi, $\geq 10^6$ copies/mL for DNA preparations, and $\geq 10^5$ $TCID_{50}$/mL for viruses. Human Metapneumovirus, Influenza A, and Influenza B viruses were spiked at $\geq 10^4$ $TCID_{50}$/mL. The organisms were tested with the GeneSTAT.MDx *Coccidioides* assay in triplicate. Initial false positive results were observed in one out of three replicates of *E. coli,* Human parainfluenzavirus 2, and *L. pneumophila*; however, no false positive results were observed for any of these panel members when an additional three replicates were tested at ten-fold higher concentrations. Three initial invalid results were observed in the study, and all three samples resolved to the expected negative result upon re-test.

Microbial interference was evaluated with the same panel tested in triplicate in the presence of *C. posadasii* spherules (strain Δcts2/Δard1/Δcts3) at 3x LoD (20.91 GEq/mL). No evidence microbial interference was observed. The results are acceptable.

Table 10. Cross Reactivity Panel

Acinetobacter baumanii	*Legionella pneumophila* [#]
Adenovirus	*Moraxella catarrhalis*
Aspergillus fumigatus	*Mycobacterium tuberculosis*
Blastomyces dermatitidis[†]	*Mycoplasma pneumoniae*
Bordetella parapertussis	*Neisseria meningitidis*
Bordetella pertussis	*Neisseria sicca*
Burkholderia cepacia	*Porphyromonas gingivalis*[†]
Candida albicans	*Proteus vulgaris*
Candida glabrata	*Pseudomonas aeruginosa*
Chlamydophila pneumoniae[†]	Respiratory Syncytial Virus
Corynebacterium diphtheriae	*Scedosporium prolificans*
Escherichia coli [#]	*Serratia marcescens*
Haemophilus influenzae	*Staphylococcus aureus (MRSA)*
Histoplasma capsulatum[†]	*Staphylococcus aureus (MSSA)*
Human Influenza A	*Staphylococcus epidermidis (MRSE)*
Human Influenza B	*Staphylococcus epidermidis (MSSE)*
Human Metapneumovirus	*Staphylococcus haemolyticus*
Human parainfluenzavirus 1	*Streptococcus agalactiae*
Human parainfluenzavirus 2[#]	*Streptococcus dysgalactiae*
Human parainfluenzavirus 3	*Streptococcus mitis*
Human parainfluenzavirus 4	*Streptococcus pneumoniae*
Klebsiella pneumoniae	*Streptococcus pyogenes*
Lactobacillus acidophilus	*Streptococcus salivarius*
Lactobacillus plantarum	

[†]Tested as purified DNA

[#] Initial false positive results were observed in one out of three replicates of these organisms; however, no false positive results were observed for any of these organisms when an additional three replicates were tested at ten-fold higher concentrations.

Interfering Substances

The susceptibility of the GeneSTAT.MDx *Coccidioides* assay to interference by a panel of 15 endogenous or exogenous substances was assessed. Interference challenge samples were prepared by spiking the test substances into BAL matrix to reach the test concentration shown in Table 11 below. *C. posadasii* spherules (strain Δcts2/Δard1/Δcts3) were added to each sample at 3x LoD (20.91 GEq/mL). No interference was observed for any substance at the tested concentrations. The results are acceptable.

Table 11. Interfering Substances Panel

Substance	Test Concentration	Substance	Test Concentration
Lidocaine HCl, 4% w/v	20% v/v	Human Genomic DNA	50 ng/mL
Levofloxacin	5 mg/mL	Albuterol Sulfate	50 µg/mL
Tobramycin	5% w/v	Mucin	1% w/v
Zanamivir	500 ng/mL	Phenylephrine	1.5% v/v
Oseltamivir	100 ng/mL	Fluticasone	5 µg/mL

Substance	Test Concentration	Substance	Test Concentration
Whole blood	2% v/v	NaCl 5%	5% w/v
Epinephrine	1 mg/mL	Nicogel, 40%	0.5% w/v
FluMist	1% v/v		

Carry-Over/ Cross-Contamination

Carry-over and cross-contamination on the GeneSTAT.MDx *Coccidioides* assay was evaluated by testing a series of high positive samples interspersed with *Coccidioides*-negative BAL samples on two GeneSTAT instruments. The high positive samples contained attenuated *C. posadasii* spherules at 1000x LoD. High positive and negative samples were alternately tested on each GeneSTAT analyzer for five cycles for a total of 20 assay runs, ten high positive, and ten negative samples overall. No evidence of amplicon carry-over or sample cross-contamination was observed, and the expected result was obtained for all samples. The results are acceptable.

g. *Assay Cut-off:*

The cut-off values for the assay *Coccidioides* result and the internal control were established and verified by analyzing contrived samples and clinical BAL/BW samples known to be positive and negative for *Coccidioides*. The cut-offs were validated by the results of the samples tested in the clinical and analytical studies.

The following decision table is used by the assay data analysis software to generate the reported assay result. This table is not displayed:

Reaction Well 1		Reaction Well 2	Reported Assay Result
Coccidioides PCR	Internal Control PCR	*Coccidioides* PCR	
Negative	Positive	Negative	*Coccidioides* DNA Not Detected
Positive	Positive	Negative	*Coccidioides* DNA Detected
Positive	Positive	Positive	*Coccidioides* DNA Detected
Positive	Negative	Positive	*Coccidioides* DNA Detected
Negative	Positive	Positive	*Coccidioides* DNA Detected
Positive	Negative	Negative	*Coccidioides* DNA Detected
Negative	Negative	Positive	INVALID
Negative	Negative	Negative	INVALID

h. *Specimen Stability*

BAL/BW sample stability was demonstrated by testing individual or pooled *Coccidioides*-negative BAL/BW clinical specimens with and without *C. posadasii* spherules spiked at three times the LoD. Samples were processed and tested with the GeneSTAT.MDx *Coccidioides* assay per the labeled instructions after storage under different conditions that included storage of neat specimens at 2-8 °C, storage at the Sputolysin digestion stage at 2-8 °C, and storage of extracted DNA at 2-8 °C and -20 °C. To support the use of frozen samples in analytical and clinical studies, neat

specimens were also tested after up to two -20 °C freeze-thaw cycles. The results of the analytical studies and the results of the prospective and retrospective clinical studies support the specimen collection and storage recommendations outlined in the labeling:

BAL/BW specimens should be maintained at 2-8 °C and must be processed at least through the Sputolysin digestion stage within seven days or less from the time of collection. Samples that have undergone Sputolysin digestion should be maintained at 2-8 °C and must be processed through the DNA extraction procedure within three days or less from Sputolysin treatment. Extracted DNA can be stored for up to eight hours at 2-8 °C or can be frozen at -20 °C or lower for up to 30 days prior to testing.

2. Comparison Studies:

   a. Clinical Comparison:

   See section L3

   b. Matrix Equivalence Study

   Not applicable

3. Clinical Studies:

   The clinical performance of the GeneSTAT.MDx *Coccidioides* assay performed on the GeneSTAT System was determined by testing prospective and retrospective samples. Due to the low prevalence of coccidioidomycosis, retrospective samples were tested to demonstrate product performance with additional positive clinical samples. All samples were tested by fungal culture followed by *Coccidioides*-specific DNA probe hybridization to confirm recovery of *Coccidioides* in positive fungal cultures (AccuProbe, Hologic, Marlborough, MA).

   Prospective Sample Testing
   A total of 236 fresh BAL/BW samples were collected prospectively from four geographically diverse regions endemic for *Coccidioides* (Phoenix, AZ, Albuquerque, NM, Tucson, AZ, and North Hollywood, CA). Samples submitted for routine fungal culture and that met the study inclusion criteria were enrolled. The samples were tested with the GeneSTAT.MDx *Coccidioides* assay prospectively at three of the sites (several samples collected from CA were sent to the NM site for testing).

   Five samples were excluded from the study due to protocol violations, sample handling violations, incomplete culture results, or insufficient specimen volume. Of the remaining 231 prospective samples that were included in the study, 189 were BAL and 42 were BW. Performance compared to fungal culture was evaluated based on results from testing BAL and BW samples combined. In comparison to fungal culture with fresh prospective specimens, the GeneSTAT.MDx *Coccidioides* assay sensitivity was 100% (3/3) and specificity was 99.6% (227/228). The GeneSTAT.MDx *Coccidioides* assay produced a false positive result in one sample. The prospective sample testing results are summarized

below for all sites combined (Table 12) and for each site individually (Table 13, Table 14, and Table 15).

Table 12. Prospective Clinical Performance - All Sites

All 3 Sites		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	3	1†	4
	NEG	0	227	227
	TOTAL	3	228	231
All 3 Sites			**95% CI**	
Sensitivity (%)		100%	43.8%	100%
Specificity (%)		99.6%	97.6%	99.9%

†One false positive result was obtained from the GeneSTAT.MDx *Coccidioides* assay at Site #2. This sample was negative for *Coccidioides* DNA when tested by an independent real-time PCR and bi-directional sequencing method.

Table 13. Prospective Clinical Performance - Site #1

Site #1		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	0	0	0
	NEG	0	92	92
	TOTAL	0	92	92
Site #1			**95% CI**	
Sensitivity (%)		*	*	*
Specificity (%)		100%	96.0%	100%

*There were no positive samples collected at Site #1.

Table 14. Prospective Clinical Performance - Site #2

Site #2		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	2	1	3
	NEG	0	50	50
	TOTAL	2	51	53
Site #2			**95% CI**	
Sensitivity (%)		100%	34.2%	100%
Specificity (%)		98.0%	89.7%	99.7%

Table 15. Prospective Clinical Performance - Site #3

Site #3		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	1	0	1
	NEG	0	85	85
	TOTAL	1	85	86
Site #3			**95% CI**	
Sensitivity (%)		100%	20.7%	100%
Specificity (%)		100%	95.7%	100%

Retrospective Sample Testing

A total of 100 frozen, de-identified BAL/BW specimens were tested at the three clinical sites. The enrolled samples included 51 positive (27 BAL and 24 BW) and 49 negative (38 BAL and 11 BW) samples. Specimens were selected from previously confirmed negative and positive samples for *Coccidioides* infection as determined by fungal culture; positive cultures were confirmed as *Coccidioides* using the AccuProbe assay. Prior to inclusion in the study, the retrospective samples were sent to a reference laboratory for confirmation of *Coccidioides* DNA status using a validated PCR assay with bi-directional sequencing. Only samples that were concordant by both culture and the reference laboratory PCR/sequencing method were included in the retrospective sample set.

The performance of the GeneSTAT.MDx *Coccidioides* assay was compared to the original culture results for this sample set. Overall positive percent agreement (PPA) was 100% (51/51) and negative percent agreement (NPA) was 95.9% (47/49). The retrospective sample testing results are summarized below for all sites combined (Table 16) and for each site individually (Table 17, Table 18, and Table 19).

Table 16. Retrospective Clinical Performance - All Sites

All 3 Sites		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	51	2[†]	53
	NEG	0	47	47
	TOTAL	51	49	100
All 3 Sites			**95% CI**	
PPA (%)		100%	93.0%	100%
NPA (%)		95.9%	86.3%	98.9%

[†] Two false positive results were obtained from the GeneSTAT.MDx *Coccidioides* assay at Site #2. These samples were negative for *Coccidioides* DNA when tested by an independent real-time PCR and bi-directional sequencing method.

Table 17. Retrospective Clinical Performance - Site #1

Site #1		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	36	0	36
	NEG	0	17	17
	TOTAL	36	17	53
Site #1			**95% CI**	
PPA (%)		100%	90.4%	100%
NPA (%)		100%	81.6%	100%

Table 18. Retrospective Clinical Performance - Site #2

Site #2		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	13	2[†]	15
	NEG	0	30	30
	TOTAL	13	32	45
Site #2			**95% CI**	
PPA (%)		100%	77.2%	100%
NPA (%)		93.8%	79.9%	98.3%

Table 19. Retrospective Clinical Performance - Site #3

Site #3		Fungal Culture / DNA Probe		
		POS	NEG	TOTAL
GeneSTAT.MDx *Coccidioides* Assay	POS	2	0	2
	NEG	0	0	0
	TOTAL	2	0	2
Site #3			**95% CI**	
PPA (%)		100%	34.2%	100%
NPA (%)		*	*	*

*There were no negative samples tested at Site #3.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The prospective study included 189 BAL and 42 BW samples that were submitted for routine fungal culture from four regions endemic for coccidioidomycosis (Phoenix, AZ, Albuquerque, NM, Tucson, AZ, and North Hollywood, CA). Among the total 231 samples, 124 were collected from males (53.7%), 102 were collected from females (44.2%), and the gender was not recorded for five samples (2.2%). Three of the samples (1.3%) were positive for *Coccidioides* by Accuprobe confirmed fungal culture, and four of the samples (1.7%) were positive by the GeneSTAT.MDx *Coccidioides* assay.

M. Instrument Name

GeneSTAT System

N. System Descriptions:

1. Modes of Operation:
   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ____X____

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X_____ or No _____

   The sponsor provided adequate verification and validation testing covering the following software/instrument components of the GeneSTAT System:

Table 20. GeneSTAT System

Component of GeneSTAT System	Descriptions of System Element
DxNA Test Module	Assay specific cartridge containing reagents, reaction wells, and associated test parameters. The analyzer reads assay specific information contained on the identification tag of Test Modules.
GeneSTAT Analyzer	Benchtop analyzer that executes mechanical, optical, fluidic actions upon the Test Module to perform PCR reactions, generate results, and transmit results to PCReports.
Firmware	Control and analysis firmware on the analyzer that runs mechanical, thermal, mixing, and optical detection functions required to execute assay protocols. The firmware processes the fluorescence signals generated and compares these values to assay specific thresholds to determine if the target has been detected.
PC	MS Windows based laptop computer locally connected via USB to the analyzer
PCReports	Software resident on the PC providing reports, user interface, and related functions.
Performance Verification Test Cartridges (PVT)	Test Modules containing functional chemistry used to periodically verify that the analyzer is functioning within acceptable limits.

3. Specimen Identification:
   The sample ID is manually entered into the GeneSTAT System during the assay run process.

4. Specimen Sampling and Handling:
   Specimens are manually prepared and transferred to the assay Test Module. Briefly, the user is required to transfer the extracted sample to the Sample Vial, attach the Sample Vial to the assay cartridge, and load the attached Sample Vial and cartridge (referred to as the Test Module) onto the GeneSTAT analyzer when prompted by the software. Subsequent specimen handling is automated.

5. Calibration:
   Calibration is not required by the user.

6. Quality Control:
   See section L1c.

O. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

Not applicable

P. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR parts 801 and 809 as well as the Special Controls for this type of device.

Q. Identified Risks to Health and Mitigation Measures:

Identified Risk	Mitigation Measures
Incorrect identification or lack of identification of a pathogenic microorganism by the device can lead to improper patient management	General Controls and Special Controls (1), (2)(i), (2)(ii), (2)(iii), (2)(iv), (2)(v), (3)(i), (3)(ii), and (3)(iii)
Failure to correctly interpret test results	General Controls and Special Controls (1), (2)(iii), (2)(iv), and (2)(v)
Failure to correctly operate the instrument	General Controls and Special Controls (1), (2)(i), (3)(ii), and (3)(iii)

R. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	• The GeneSTAT.MDx *Coccidioides* Assay is the first assay to use a qualitative real-time polymerase chain reaction (PCR) to detect and identify *Coccidioides spp.* nucleic acids directly from patient bronchial alveolar lavage specimens and bronchial wash specimens. • The GeneSTAT.MDx *Coccidioides* Assay can provide results from an extracted sample in approximately 1.5 hours, compared to traditional fungal culture which may take up to three weeks to return results. The quicker time to result may allow for more rapid initiation of antifungal therapy, which may result in improved patient outcomes with decreases in morbidity or mortality. • Compared to traditional fungal culture, the GeneSTAT.MDx *Coccidioides* Assay demonstrated sensitivity/PPA of 100% in both prospective and retrospective studies. Specificity/NPA was 99.6% and 95.9% in the prospective and retrospective studies respectively. The performance in the prospective and retrospective clinical studies suggests that the GeneSTAT.MDx *Coccidioides* Assay will be highly sensitive and specific for the diagnosis of coccidioidomycosis.
Summary of the Risk(s)	• False positive results, and false negative results are the primary risks associated with use of the GeneSTAT.MDx *Coccidioides* Assay. • A false positive result may lead to unnecessary antifungal therapy, with associated adverse events, such as potential allergic reactions. • A false negative result may result in a delay of antifungal therapy, with subsequent worsening of infection and associated increase in morbidity or mortality.
Summary of Other Factors	None.

Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of the GeneSTAT.MDx *Coccidioides* Assay outweigh the potential risks in light of the listed special controls and applicable general controls. The GeneSTAT.MDx *Coccidioides* Assay is the first assay to use a qualitative real-time polymerase chain reaction to detect and identify *Coccidioides spp.* nucleic acids directly from bronchial alveolar lavage specimens and bronchial wash specimens and is likely to benefit patients by more rapidly diagnosing coccidioidomycosis. The excellent performance observed during the clinical trials in comparison to traditional fungal culture and the proposed special controls suggest that errors will be uncommon and will be well mitigated by current laboratory practices, which includes standard of care fungal culture and product labelling. The GeneSTAT.MDx *Coccidioides* Assay could provide substantial benefits as an aid in the diagnosis of coccidioidomycosis.

S. Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3376. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QAA

Device Type: Device to detect and identify fungal nucleic acids directly in respiratory specimens

Class: II (special controls)

Regulation: 21 CFR 866.3376

(a) Identification. A device to detect and identify fungal nucleic acids directly in respiratory specimens is an in vitro diagnostic device intended for the detection and identification of fungal pathogens in respiratory specimens collected from patients with signs or symptoms and suspicion of fungal infection. The device is intended to aid in the diagnosis of fungal disease in conjunction with clinical signs and symptoms and other laboratory findings.

(b) Classification. Class II (special controls). A device to detect and identify fungal nucleic acids directly in respiratory specimens must comply with the following special controls:

(1) The intended use for the 21 CFR 809.10 compliant labeling must include a detailed description of what the device detects, the type of results provided to the user, the clinical indications appropriate for test use, and the specific population(s) and testing location(s) for which the device is intended.

(2) The 21 CFR 809.10 compliant labeling must include:

(i) A detailed device description, including the device components, instrument requirements, ancillary reagents required but not provided, and a detailed explanation of the methodology including all pre-analytical methods for processing of specimens.

(ii) Performance characteristics from analytical studies, including but not limited to analytical sensitivity (Limit of Detection), inclusivity, reproducibility, interference, cross reactivity, interfering substances, carryover/cross contamination, and specimen stability.

(iii) A statement that the device is intended to be used in conjunction with clinical history, signs and symptoms and the results of other diagnostic tests.

(iv) A detailed explanation of the interpretation of test results and acceptance criteria for any quality control testing.

(v) A limiting statement that negative results do not preclude the possibility of infection, and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

(3) The compliant design controls must include:

(i) Performance characteristics from clinical studies that include prospective (sequential) samples and, if appropriate, additional characterized samples. The study must be performed on a study population consistent with the intended use population and compare the device performance to results obtained from well-accepted comparator methods. Documentation from the clinical studies must include the clinical study protocol (including predefined statistical analysis plan), clinical study report, and results of all statistical analyses.

(ii) A detailed device description of the following:

(A) Overall device design including all device components and all control elements incorporated into the testing procedure

(B) Thorough description of the methodology including, but not limited to, primer/probe sequences, primer/probe design and rationale for target sequence selection

(C) Computational path from collected raw data to reported result (e.g., how collected raw signals are converted into a reported signal and result), as applicable

(iii) A detailed documentation for device software, including, but not limited to, software applications and hardware-based devices that incorporate software.