Statement of Deficiencies	**(X1) Provider/Supplier/CLIA Identification Number**	**(X3) Date Survey Completed**
	21D2264828	08/02/2023
Name of Provider or Supplier	**Street Address, City, State**	
John G Deleonibus Dpm Pa	2086 Generals Hwy #101, Annapolis, MD	

For information on the provider's plan to correct this deficiency, please contact the provider or the state survey agency.

(X4) ID Prefix Tag	Summary Statement of Deficiencies
D3011	FACILITIES CFR(s): 493.1101(d) Safety procedures must be established, accessible, and observed to ensure protection from physical, chemical, biochemical, and electrical hazards, and biohazardous materials. This STANDARD is not met as evidenced by: Based on surveyor observation and interview with the general supervisor (GS), the laboratory did not ensure that an eye wash station was located in the laboratory area where testing occurs. Findings: 1. During a tour of the laboratory at 1:30 PM, it was observed that there was no eye wash station available in the laboratory where laboratory testing is performed. 2. A wall-mounted "Eye Wash Safety Station" was present in another room adjacent to the laboratory, however there were no bottles of eye wash solution in the holder. 3. This was confirmed by the GS during an interview on 08/02/2023 at 1:30 PM.
D5211	EVALUATION OF PROFICIENCY TESTING PERFORMANCE CFR(s): 493.1236(a) The laboratory must review and evaluate the results obtained on proficiency testing performed as specified in subpart H of this part. This STANDARD is not met as evidenced by: Based on review of proficiency testing (PT) records and interview with the general supervisor (GS), the laboratory failed to document the review and evaluation of PT results. Findings: 1. The laboratory performed molecular wound panel and molecular fungal panel testing. 2. The laboratory was enrolled with the American Proficiency Institute (API) for molecular microbiology beginning in 2023 and performed

	alternative split sample PT in 2022. 3. The performance evaluation for the API 2023 1st Microbiology PT event was not signed and dated as reviewed by the laboratory director (LD) or designee. 4. The fungal and wound panel split sample testing events from 09/2022 were signed as reviewed by the technical supervisor on 01/31/2022. 5. The fungal panel split sample testing event number 092022 showed that sample F-722 was expected to be detected for Microsporum canis, sample F-822 was expected to be detected for Trichophyton anthropophilic species, and sample F-922 was expected to be detected for Pseudomonas aeruginosa. None of above targets were detected by the laboratory. 6. The wound panel split sample testing event number 122022 showed that sample U-322 was expected to be detected for Klebsiella aerogenes but was not detected by the laboratory. 7. There was no documentation that an investigation into the root cause of the failed split sample results was performed and corrective actions taken, if indicated. 8. During the survey on 08/02/2023 at 4:00 PM, the GS confirmed that the API PT evaluations were not documented as reviewed by the LD or designee and there was no documentation of the investigation into and corrective actions taken for failed split sample PT results.
D5215	EVALUATION OF PROFICIENCY TESTING PERFORMANCE CFR(s): 493.1236(b)(2) The laboratory must verify the accuracy of any analyte, specialty or subspecialty assigned a proficiency testing score that does not reflect laboratory test performance (that is, when the proficiency testing program does not obtain the agreement required for scoring as specified in subpart I of this part, or the laboratory receives a zero score for nonparticipation, or late return or results). This STANDARD is not met as evidenced by: Based on review of the proficiency testing (PT) records and interview with the general supervisor (GS), the laboratory failed to perform a self-evaluation of ungraded PT results. Findings: 1. The performance evaluation for the 2023 1st Microbiology PT event showed that results for the gene targets vanB, CTX-M, KPC, mecA, NDM, and vanA were "Not Graded" by the PT provider. 2. There was no documentation that the ungraded PT results were compared with the results listed in the PT provider's data summary. 3. During the survey on 08/02/2023 at 4:00 PM, the GS confirmed that there was no documentation of the laboratory's self-evaluation of ungraded PT results for the 2023 1st Microbiology PT event.
D5217	EVALUATION OF PROFICIENCY TESTING PERFORMANCE CFR(s): 493.1236(c)(1) At least twice annually, the laboratory must verify the accuracy of any test or procedure it performs that is not included in subpart I of this part. This STANDARD is not met as evidenced by: Based on review of the test menu, review of the proficiency testing (PT) provider's catalog, and interview with the outside laboratory consultant (OLC), the laboratory failed to ensure that all gene targets for the molecular wound and fungal panel testing were verified for accuracy at least twice annually. Findings: 1. The laboratory performed two molecular assays: 1) a wound panel that tested for 29 pathogen and 9 antibiotic resistance gene targets and 2) a fungal panel that tested for 20 pathogen and 1 antibiotic resistance gene targets. 2. The laboratory enrolled with American

	Proficiency Institute (API) in PT modules for molecular blood and nail fungus panels. All targets included in each API module was listed in the 2023 Catalog of Programs (catalog). 3. The following gene targets from the wound panel were not included in the API catalog: Citrobacter freundii/braakii, C. koseri, Mycoplasma genitalium, M. hominis, Prevotella bivia, Staphylococcus saprophyticus, and Ureaplasma urealyticum. 4. The following gene targets from the fungal panel were not included in the API catalog: Sarocladium strictum. 5. The following antibiotic resistance gene targets were not included in the API catalog: dfrA, qnr, and sul. 6. During the survey on 08/02/2023 at 4:00 PM, the OLC confirmed that not all gene targets from the molecular wound and fungal panels were included in the API PT modules and the accuracy of those gene targets would not be verified twice annually without enrolling in additional modules or performing alternative PT.
D5311	SPECIMEN SUBMISSION, HANDLING, AND REFERRAL CFR(s): 493.1242(a) The laboratory must establish and follow written policies and procedures for each of the following, if applicable: (1) Patient preparation. (2) Specimen collection. (3) Specimen labeling, including patient name or unique patient identifier and, when appropriate, specimen source. (4) Specimen storage and preservation. (5) Conditions for specimen transportation. (6) Specimen processing. (7) Specimen acceptability and rejection. (8) Specimen referral. This STANDARD is not met as evidenced by: I. Based on review of the procedure and interview with the general supervisor (GS), the laboratory failed to establish procedures for receipt and accessioning of patient specimens for the molecular wound and fungal panel testing. Findings: 1. The laboratory performed a molecular wound panel and a molecular fungal panel assay on specimens received from their own practice. 2. The GS stated that when the specimens were received into the laboratory, they were given accession numbers based on the initials of the patient's first and last name and their birthdate. 3. The specimens were then assigned sample numbers based on whether they were to be tested on the wound or fungal panel or both. 4. On 08/02/2023 at 10:00 AM, the GS confirmed that the laboratory did not have a procedure describing how the specimens were received into the laboratory, accessioned, and assigned sample numbers. II. Based on review of procedures, stability studies, and final patient reports and interview with the general supervisor (GS) and the outside laboratory consultant (OLC), the laboratory failed to ensure that specimen stability defined in the procedures matched the study data, that stability studies used the same specimen matrix as what was received and tested in the laboratory, that acceptability criteria and specimen preparation for the stability studies were described, and that specimens were tested within the determined stability limits for molecular wound and fungal panel testing. Findings: 1. The laboratory performed two molecular assays: 1) a wound panel that tested for 29 pathogen and 9 antibiotic resistance gene targets and 2) a fungal panel that tested for 20 pathogen and 1 antibiotic resistance gene targets. 2. The wound panel specimen stability stated in the procedure did not match the data from the stability study: a. The procedure for the wound panel stated, "If the specimen will be processed (nuclear extraction) for analysis within 5 days after collection, keep it at room temperature (20-25C) or refrigerate at 4-8C" and if "the analytical system fails, processed patient samples will be stored at [less than or equal to] -20C until analytical systems are operational within 21 days." b. The stability study stated the specimens were "tested over three days at room temperature." c. Table 4 of the stability study

	showed a summary of results for Day 0, Day 1, and Day 2. d. Table 5 showed the raw data which included run 1 (Day 0 results) and run 2 (Day 1 results). The raw data for Day 2 was not included. e. There was no stability data for Day 3 or for refrigerated or frozen temperatures. 3. The fungal panel specimen stability stated in the procedure did not match the data from the stability study: a. The procedure for the fungal panel stated, "Samples are stable in transport media or dry for up to five days after collection at room temperature." b. The stability study stated that "A nail specimen was spiked with P. aeruginosa and C. albicans to determine if the sample could be stored for up to 3 days post collection." c. Table 4 of the stability study showed a summary of results for Day 0, Day 1, and Day 2. There was no data from Day 3. d. The stability study did not specify if the nail sample was in transport media or dry. 4. Study data showed that specimen stability for the wound and fungal panels was determined to be two days. Wound sample 4 was collected and received on 07/19 /2023 and was tested on 7/24/2023, 5 days after collection. Fungal sample 3 was collected and received on 07/18/2023 and was tested on 07/24/2023, 6 days after collection. 5. The GS stated that all wound specimens received and tested were collected with Copan eSwabs. The stability study stated that a "contrived urine specimen was spiked with the nucleic acid of several targets" and the positive sample was labeled as "UTI [urinary tract infection] + Stability." There was no stability data for wound swabs containing whole pathogen. 6. Neither the stability study for the wound panel nor fungal panel included the acceptability criteria for change in cycle threshold (Ct) values. The stability study for the fungal panel showed that Pseudomonas aeruginosa had a Ct value change of -3.78 from Day 0 to Day 1 and -4.40 from Day 0 to Day 2. There was no discussion of whether this was within the acceptability criteria or indicated a risk of a false positive result if not tested on the date of collection. 7. The stability study summary data (Table 4) for the wound panel included only 2 of the 29 possible pathogen targets and for the fungal panel included only 2 of the 20 possible pathogen targets. Neither study looked at the antibiotic resistance genes. At 10:15 AM on 08/02/2023, the OLC stated that P. aeruginosa and Candida albicans were chosen for the fungal stability study because they were hardier. There was no discussion or data for stability of less hardy pathogens. 8. Neither the stability study for the wound panel nor fungal panel described what pathogens were actually spiked into the specimens. Table 4 showed the summary data for 2 pathogens (see #7 above). The raw data showed pathogens with Ct values on Day 0 below their cutoff values: a. The antibiotic resistance gene dfrA had a cutoff Ct value of 33.54. The wound study showed dfrA had Ct 21.77 on Day 0 and no Ct value on Day 1. b. Candida glabrata had a cutoff Ct value of 34.75. The fungal study showed C. glabrata had Ct 34.08 on Day 0, Ct 38.96 on Day 1, and Ct 37.37 on Day 2. c. The wound study showed the endogenous control (EC) had Ct 31.13 on Day 0 and no Ct value on Day 1. The fungal study showed no EC Ct value on Days 0-2. d. There was no discussion on whether these three targets were expected to be detected and if not, why they had Ct values below the cutoff. 9. All results for the positive control on the fungal panel stability study showed no Ct value. 10. During the survey on 08/02/2023 at 4:00 PM, the OLC confirmed that the stability listed in the procedures was not consistent with the study data, the wound stability data stated it was from urine matrix not a wound swab, stability was only assessed for 2 pathogens from each panel, no acceptability criteria was defined, changes in Ct values for the fungal panel were not discussed, and specimens were tested beyond their stability as determined by the stability studies.
D5400	ANALYTIC SYSTEMS CFR(s): 493.1250

	Each laboratory that performs nonwaived testing must meet the applicable analytic systems requirements in 493.1251 through 493.1283, unless HHS approves a procedure, specified in Appendix C of the State Operations Manual (CMS Pub.7), that provides equivalent quality testing. The laboratory must monitor and evaluate the overall quality of the analytic systems and correct identified problems as specified in 493.1289 for each specialty and subspecialty of testing performed. This CONDITION is not met as evidenced by: Based on record review and interview with the general supervisor and the outside laboratory consultant, the laboratory failed to ensure that the GS had access to and was trained on the approved procedure for performing the molecular fungal panel assay (D5401); failed to include instructions in the procedure manual for how to evaluate the endogenous control (EC) for the molecular fungal panel testing (D5403 I); failed to include instructions in the procedure manual for performing corrective actions for failed positive quality control (QC) results on patients tested since the last time the positive QC was successful (D5403 II); failed to include instructions in the procedure manual for verifying that the weekly calibration plate results fell within acceptable ranges and what corrective actions to take if results fell outside of acceptable ranges (D5403 III); failed to ensure that the laboratory's procedure for evaluating and interpreting results for the molecular fungal panel assay reflected laboratory practice (D5403 IV); failed to define the expiration date for extraction kits and document when each lot number of extraction kits and amplification plates were put into use for molecular wound and fungal testing (D5417); failed to ensure that the validation study summaries described how all performance specifications were determined (D5423); failed to run 2 levels of QC each day of patient testing and failed to establish an Individualized Quality Control Plan (IQCP) based on accurate data (D5445); failed to run a negative extraction control for the molecular wound and fungal panel testing (D5453); failed to ensure the EC was performed for the molecular fungal panel testing (D5455); and failed to document all activities completed for the risk assessment (RA) in the laboratory's IQCP, including data to support their RA decisions (D5481).
D5401	PROCEDURE MANUAL CFR(s): 493.1251(a) A written procedures manual for all tests, assays, and examinations performed by the laboratory must be available to, and followed by, laboratory personnel. Textbooks may supplement but not replace the laboratory's written procedures for testing or examining specimens. This STANDARD is not met as evidenced by: Based on review of the procedure and interview with the general supervisor (GS), the laboratory failed to ensure that the GS had access to and was trained on the approved procedure for performing the molecular fungal panel assay. Findings: 1. The laboratory performed a molecular fungal panel assay that tested for 20 fungal pathogens and 1 antibacterial resistance gene. The master mix reagents for each target as well as a positive, negative, and endogenous control (EC) were designated to specific wells on a plate. 2. The laboratory submitted a copy of the fungal panel procedure that was approved by the laboratory director on 07/25/2022 to the surveyors prior to the survey (approved procedure). 3. The approved procedure included a plate map showing that all fungal panel targets were located within two columns. Column 1

	included targets in wells A-H and column 2 included targets in wells A-D. The EC was located in column 2, well D. 4. The approved procedure stated to pipette the samples into wells A-H of the first column and wells A-D of the second column. 5. The test procedure that was located in the laboratory, that the GS was trained on and followed (lab procedure), stated to pipette samples into wells A-B of the second column and included two plate map images. "Figure 1: Plate Map Fungal Panel on CFX 96 or CFX Opus" matched the lab procedure and showed that the EC was located in column 2, well B. "Figure 3. Plate Editor Screen" showed that samples should be pipetted into column 2 wells A-C and that the EC was located in column 2, well C. 6. During the survey on 08/02/2023 at 3:50 PM, the GS confirmed that the procedure in the laboratory was not the approved procedure sent to the surveyors prior to the survey.
D5403	PROCEDURE MANUAL CFR(s): 493.1251(b) The procedure manual must include the following when applicable to the test procedure: (1) Requirements for patient preparation; specimen collection, labeling, storage, preservation, transportation, processing, and referral; and criteria for specimen acceptability and rejection as described in 493.1242. (2) Microscopic examination, including the detection of inadequately prepared slides. (3) Step-by-step performance of the procedure, including test calculations and interpretation of results. (4) Preparation of slides, solutions, calibrators, controls, reagents, stains, and other materials used in testing. (5) Calibration and calibration verification procedures. (6) The reportable range for test results for the test system as established or verified in 493.1253. (7) Control procedures. (8) Corrective action to take when calibration or control results fail to meet the laboratory's criteria for acceptability. (9) Limitations in the test methodology, including interfering substances. (10) Reference intervals (normal values). (11) Imminently life-threatening test results, or panic or alert values. (12) Pertinent literature references. (13) The laboratory's system for entering results in the patient record and reporting patient results including, when appropriate, the protocol for reporting imminently life threatening results, or panic, or alert values. (14) Description of the course of action to take if a test system becomes inoperable. This STANDARD is not met as evidenced by: I. Based on review of the procedure and interview with the general supervisor (GS) and outside laboratory consultant (OLC), the laboratory's procedure failed to include instructions for how to evaluate the endogenous control (EC) for the molecular fungal panel testing. Findings: 1. The laboratory performed a molecular fungal panel that tested for 20 fungal pathogens, 1 antibacterial resistance gene, a positive and negative control, and an EC that was used as an extraction and inhibition control. 2. The approved procedure stated that the "endogenous control must show amplification to pass" and that if "all controls pass, the patient results can be reviewed and released." 3. The GS stated that they were verbally told that they could release patient results with a negative EC if other targets in the panel tested positive. 4. During the survey on 08/02/2023 at 4:00 PM, the GS and OLC confirmed that patient results for the molecular fungal assay could be released if the EC was negative and other targets tested positive and that this was not stated in the approved procedure. II. Based on review of the procedure and interview with the outside laboratory consultant (OLC), the laboratory's procedure failed to include instructions for performing corrective actions for failed positive quality control (QC) results on patients tested since the last time the positive QC was successful. Findings: 1. The laboratory performed two

	molecular assays: 1) a wound panel that tested for 29 pathogen and 9 antibiotic resistance gene targets and 2) a fungal panel that tested for 20 pathogen and 1 antibiotic resistance gene targets. 2. The laboratory ordered plates from an outside manufacturer for each test panel that were pre-filled with the master mixes for each gene target in designated wells. A single lot number of plates could last multiple weeks. 3. Every week the laboratory tested a positive QC sample for each panel that contained the targets for each pathogen and antibiotic resistance gene found in the panel. 4. The procedure for both the wound and fungal panel molecular assays stated that "The weekly positive QC run is evaluated to ensure amplification occurred for each target. This is noted on a checklist and is kept on file. If amplification did not occur for any target, troubleshooting of the testing must occur before running patient samples." 5. The procedure did not include instructions for what corrective actions should be taken for patient specimens that were run on the same lot number of plates since the last time the positive QC amplified as expected, if applicable. 6. During the survey on 08/02/2023 at 4:00 PM, the OLC confirmed that there were no procedures stating what corrective actions should be taken for patients tested on the same lot number of wound panel or fungal panel plates that had failed positive QC results since the last time the positive QC was successfully performed. III. Based on review of the procedure and final report for the molecular wound panel assay and interview with the general supervisor (GS), the laboratory's procedure failed to include instructions for verifying that the weekly calibration plate results fell within acceptable ranges and what corrective actions to take if results fell outside of acceptable ranges. Findings: 1. The molecular wound panel was semi-quantitative with results reported as Low (less than 10,000 colony forming units per milliliter (CFU/ml), Medium (50,000-100,000 CFU/ml), or High (greater than 100,000 CFU/ml). 2. The procedure stated "The Detected value is in ranges based on the Ct [cycle threshold] values based on the following: Low - ((Ct-3)-Ct; Medium - ((Ct-6)-(Ct-3.1)); High - [less than or equal to] (Ct-6.1)." Though not included in the procedure, the GS referred to tables titled "Cycle Threshold (CT) Ranges" that listed the acceptable ranges for all targets for the Low, Medium, and High ranges to evaluate which range the Ct values fell into. 3. All gene targets were confirmed on a positive control calibration plate (QC plate) that was tested weekly. 4. The GS stated that they compared Ct results for each gene target from the weekly QC plate to the values listed in the Low range. 5. The procedure did not state whether the weekly QC sample was Low, Medium, or High and did not give instructions for corrective actions to take if the QC values did not fall within the correct Ct range. IV. Based on review of the procedure and interview with the general supervisor (GS) the laboratory's procedure for evaluating and interpreting results for the molecular fungal panel assay did not reflect laboratory practice. Findings: 1. The fungal panel procedure stated "If there is a result (Ct value) lower than the pathogen cutoff value (as determined by the validations), the patient is said to be Detected for that pathogen. The Detected value is in ranges based on the Ct [cycle threshold] values based on the following" and listed ranges for Low, Medium, and High. 2. The chart titled "Cycle Threshold (CT) Ranges" listed the acceptable ranges for all targets for the Low, Medium, and High ranges. 3. The GS stated that the results from the fungal panel were not reported as Low, Medium, and High, but as detected and not detected and patient Ct results were reported as detected if below the cutoff value or not detected if above the cutoff value. 4. The procedure for the molecular fungal panel assay did not reflect laboratory practice for evaluating patient results.
D5417	TEST SYSTEMS, EQUIPMENT, INSTRUMENTS, REAGENT CFR(s): 493.1252(d) Reagents, solutions, culture media, control materials, calibration materials, and other

	supplies must not be used when they have exceeded their expiration date, have deteriorated, or are of substandard quality. This STANDARD is not met as evidenced by: Based on review of the procedure and manufacturer's instructions for use (IFU), observation, and interview with the outside laboratory consultant (OLC), the laboratory failed to define the expiration date for extraction kits and document when each lot number of extraction kits and amplification plates were put into use for molecular wound and fungal testing. Findings: 1. The laboratory performed molecular wound and fungal panel testing which included an extraction process to isolate nucleic acid from patient specimens. The extracted nucleic acid was then added to plates that were pre-filled with master mixes for each gene target tested on the panels. 2. All extraction reagents and supplies were received in a single kit that was assigned a lot number. 3. The expiration date for the extraction kit lot numbers was not defined on the box, the manufacturer's IFU, or in the laboratory's procedure. 4. At 12:40 PM on 08/02/2023, the OLC stated that the manufacturer advised them that the expiration date was 1 year from the date the laboratory received the extraction kits. It was observed at that time that all kits stored in the laboratory were labeled with the date of receipt and an expiration date that was 1 year from the receipt date. 5. The plates used for both the wound and fungal panels expired 6 months after receipt and were labeled accordingly on the box. 6. The laboratory did not document the dates that each lot number was put into use for patient testing to be able to track which reagents and supplies were used for each testing batch. 7. During the survey on 08/02/2023 at 4:00 PM, the OLC confirmed that the expiration date for the extraction kits was not defined and that the dates that each lot number of extraction kits and test plates was put into use was not documented.
D5423	ESTABLISHMENT AND VERIFICATION OF PERFORMANCE CFR(s): 493.1253(b)(2) Each laboratory that modifies an FDA-cleared or approved test system, or introduces a test system not subject to FDA clearance or approval (including methods developed in-house and standardized methods such as text book procedures), or uses a test system in which performance specifications are not provided by the manufacturer must, before reporting patient test results, establish for each test system the performance specifications for the following performance characteristics, as applicable: (2)(i) Accuracy. (2)(ii) Precision. (2)(iii) Analytical sensitivity. (2)(iv) Analytical specificity to include interfering substances. (2)(v) Reportable range of test results for the test system. (2)(vi) Reference intervals (normal values). (2)(vii) Any other performance characteristic required for test performance. This STANDARD is not met as evidenced by: I. Based on review of the procedure and the validation summary and interview with the general supervisor (GS) and outside laboratory consultant (OLC), the laboratory failed to ensure that the data presented in the molecular wound panel validation was for wound specimens and not urine specimens. Findings: 1. The "Specimen Collection" section of the molecular wound panel procedure stated that acceptable specimens were specimens collected using a Copan eSwab, by aspiration with sterile saline, and by curettage. The GS confirmed that only swab specimens were received and tested. 2. The "Background" section of the validation summary stated, "This validation aims to establish that the PCR assays...may be satisfactorily used as a

diagnostic tool to assess human specimens for urinary tract infections and the BioRad CFX 96." 3. The "Interfering Substances" section of the validation summary stated, "Common substances found in urine matrix was introduced into the specimen, including commercially available clean blood, vaginal fluid, and seminal fluid." The raw data for this section was for "Urine", "Urine + vaginal", and "Urine + Blood." 4. The "Stability Study" section of the validation summary stated, "A contrived urine specimen was spiked with the nucleic acid of several targets" and the raw data was labeled as "UTI [urinary tract infection] Stability." 5. The validation summary made no mention that specimens collected via swab, aspiration, and curettage were used to establish performance specifications for the molecular wound panel assay. 6. During the survey on 08/02/2023 at 4:00 PM, the OLC confirmed that the validation summary stated that the validation studies were performed on urine specimens not wound specimens collected by swab, aspiration, and curettage. II. Based on review of the validation summaries and procedures and interview with the outside laboratory consultant (OLC), the laboratory failed to ensure that the validation summaries for the molecular wound and fungal panel assays described how the performance specifications listed in the precision and limit of detection (LOD) section were determined. Findings: 1. The laboratory performed two molecular assays: 1) a wound panel that tested for 29 pathogen and 9 antibiotic resistance gene targets and 2) a fungal panel that tested for 20 pathogen and 1 antibiotic resistance gene targets. 2. The "Precision and Limit of Detection" section from the validation summaries of both panels stated, "The precision and limit of detection experiments were performed by spiking the assays with approximately 100 copies/[microliter] of genomic nucleic acid that was quantified by spectrophotometry for each target" and that "Six positive replicates and six negative replicates were tested each day over four days." 3. The validation summaries did not specify what specimen matrix was used to spike the nucleic acid into. 4. The data for each gene target was summarized and showed that the study was performed over 3 days not 4 days, as stated. 5. The validation summaries did not specify if 100 copies/microliter was the lower limit of detection (LLOD) for all targets in each panel, how the LLOD was determined, and how the cycle threshold (Ct) values from the LLOD was used to define the Ct cutoff value. a. Both the wound and fungal panel procedures stated, " ...multiple serial dilutions were run to establish a standard curve. A correlation was developed using Pathogen PFU /CFU with DNA copy number. The copy number derived from this correlation established the Final Concentration for the Lower Limit of Detection for each pathogen." Neither validation summary included data from serial dilutions to determine the LLOD. b. The validation summaries stated "from this data, we derived the absolute value for each pathogen. This absolute value serves as the cutoff for the respective organism." c. There was a separate chart for each gene target listing the Ct values from the 6 replicates for the LLOD and their mean. d. It was not specified how the cutoff was calculated. For example, the data for the antibiotic resistance gene target blaKPC showed a mean Ct value of 28.13 for the 6 LLOD replicates. The absolute value to be used as the cutoff was listed as Ct 33.28. The formula used to determine the cutoff was not described. 6. The validation summaries did not describe how the defined acceptable Ct ranges for each gene target were established and how frequently they need to be calibrated. a. The charts titled "Cycle Threshold (CT) Ranges" defined 3 acceptable Ct ranges for each gene target: Low, Medium, and High. b. Neither the validation summaries nor the procedures defined what Low, Medium, and High represented. The final report for the wound panel showed that Low was for less than 10,000 colony forming units per milliliter (CFU/ml), Medium was for 50,000-100,000 CFU/ml, and High was for greater than 100,000 CFU/ml. c. The validation summaries did not describe how the Low, Medium, and High Ct value ranges were determined, how they were verified, and how frequently they need to be

calibrated to ensure the Ct ranges remain accurate. 7. During the survey on 08/02/2023 at 4:00 PM, the OLC confirmed that the precision data was collected over 3 days, not 4, and that the validation summaries did not describe how the LLOD and Ct value ranges were determined and validated. III. Based on review of the validation summaries and interview with the outside laboratory consultant (OLC), the laboratory failed to ensure that the validation summaries for the molecular wound and fungal panel assays described how the performance specifications listed in the cross-reactivity section were determined and included a discussion of discordant results. Findings: 1. The laboratory performed two molecular assays: 1) a wound panel that tested for 29 pathogen and 9 antibiotic resistance gene targets and 2) a fungal panel that tested for 20 pathogen and 1 antibiotic resistance gene targets. 2. The validation summaries for both assays stated, "Cross-reactivity studies were conducted by comparing the result of the contrived specimens, each well containing specific primer-probe combinations with appropriate buffers were introduced" and that "No cross-reactivity was observed." 3. Table 1 presented the data for 6 samples by listing the name of each gene target and what the expected cycle threshold (Ct) values and obtained Ct values were for each sample. 4. The validation summaries did not describe the components of each of the 6 contrived specimens. 5. In Table 1 the detected gene targets for each specimen were highlighted in either green for when the obtained Ct matched the expected Ct or red for results that didn't match. Both assays obtained Ct vales that were not expected: a. Results found in Table 1 for the wound panel were given as a Ct value. It was not stated where the expected Ct value came from. b. The obtained Ct value for blaKPC was 30.75 for sample 5 and 32.63 for sample 6. Both samples were not expected to be detected. c. The obtained Ct value for Citrobacter koseri was 30.97 for sample 6. The sample was not expected to be detected. d. The obtained Ct value for Pseudomonas aeruginosa was 32.48 for sample 6. The sample was not expected to be detected. e. The obtained Ct value for Proteus mirabilis was 32.81 for sample 5. The sample was not expected to be detected. f. The obtained Ct value for Staphylococcus epidermidis was 32.25 for sample 5. The sample was not expected to be detected. g. Results found in Table 1 for the fungal panel were given as Detected or Not Detected. h. Candida glabrata was detected in sample 3 when it was not expected to be detected. i. Candida kruseii was detected in sample 2 when it was not expected to be detected. j. Pseudomonas aeruginosa was not detected in sample 5 when it was expected to be detected. k. Candida parapsilosis was not detected in sample 2 when it was expected to be detected. l. Trichophyton anthropophilic species was detected in sample 6 when it was not expected to be detected. m. The endogenous control was detected in sample 6 when it was not expected to be detected. n. The validation summary did not discuss why these values were highlighted in red or why it was determined that no cross-reactivity was observed when unexpected values were obtained. 6. The endogenous control (EC) was a human housekeeping gene and was used as an extraction and inhibition control. The validation summaries did not discuss why the EC was not detected in samples 1-5 for both the wound and fungal panels. 7. Cross-reactivity was not performed for Mycoplasma genitalium, Scytalidium dimidiatum, and Sarocladium strictum. 8. The OLC stated that the sequences for all the primers and probes were run through the Basic Local Alignment Search Tool (BLAST) to verify specificity of the individual sequences. A summary of the BLAST search data was not included in the validation documentation. 9. During the survey on 08/02/2023 at 4:00 PM, the OLC confirmed that the validation studies did not address the discordant cross-reactivity results and the BLAST search data. IV. Based on review of the validation summary and interview with the outside laboratory consultant (OLC), the laboratory failed to ensure that the interfering substances section of the validation summary for the molecular wound panel included accurate data and for the molecular fungal panel included acceptability

	criteria. Findings: 1. The laboratory performed two molecular assays: 1) a wound panel that tested for 29 pathogen and 9 antibiotic resistance gene targets and 2) a fungal panel that tested for 20 pathogen and 1 antibiotic resistance gene targets. 2. The OLC confirmed that the interfering substances data for the wound panel was for urine specimens not wound specimens. Cross refer to D5423 I for details. 3. The laboratory tested nail clippings for the molecular fungal panel. The "Interfering Substances" section of the validation summary stated, "Tolnaftate was added to a fungal sample to determine if it was inhibitory." 4. Table 2 gave a summary of the "Fungal" and "Fungal and Tolnaftate" results. 5. The validation summary did not describe what the fungal sample matrix consisted of, how much Tolnaftate was added to the sample, how long after adding Tolnaftate the samples were tested, and whether the Fungal and Fungal plus Tolnaftate samples were prepared and tested at the same time. 6. The acceptability criteria was not defined. 7. Candida kruseii had a "Fungal" Ct value of 36.03, which was beyond the Ct cutoff value of 33.81, and a "Fungal and Tolnaftate" Ct value of 31.18. There was no discussion of why the Ct shift may have occurred or if there was a risk of false positive results for Candida kruseii. 8. During the survey on 08/02/2023, the OLC confirmed that the shift in Ct value was not discussed.
D5445	CONTROL PROCEDURES CFR(s): 493.1256(d)(1)(2)(g) Unless CMS Approves a procedure, specified in Appendix C of the State Operations Manual (CMS Pub. 7), that provides equivalent quality testing, the laboratory must--(d)(1) Perform control procedures as defined in this section unless otherwise specified in the additional specialty and subspecialty requirements at 493.1261 through 493.1278. (d)(2) For each test system, perform control procedures using the number and frequency specified by the manufacturer or established by the laboratory when they meet or exceed the requirements in paragraph (d)(3) of this section. (g) The laboratory must document all control procedures performed. This STANDARD is not met as evidenced by: Based on procedure manual and quality control (QC) record review and interview with the general supervisor (GS) and outside laboratory consultant (OLC), the laboratory did not run 2 levels of QC each day of patient testing and failed to establish an Individualized Quality Control Plan (IQCP) based on accurate data. Findings: 1. The laboratory performs wound and fungal testing using Real-Time Reverse transcriptase Quantitative Polymerase Chain Reaction (RT-QPCR). The laboratory's test menu includes targets for 29 wound organisms, 20 fungal organisms, and 9 antibiotic resistance genes. 2. During an interview at 11:15 AM on the day of the survey, the GS stated that the laboratory has been running QC "weekly" since they began working at the laboratory in June, 2022. QC record review showed that the laboratory failed to run a positive and a negative control for each of the targets listed on the test menu each day of patient testing. 3. A review of the IQCP showed that it was signed and approved by the current technical supervisor and the laboratory director on 06/08/2023, however during an interview the GS stated that the laboratory began testing in August 2022. 4. Review of the IQCP showed that on the "Table of Contents" it stated, "SCOPE: This document pertains only to and is limited to the quality control of molecular laboratory developed tests performed on the QuantStudio3 real time PCR platform." The laboratory performs testing using a BioRad CFX 96 Real Time System, not a "QuantStudio3 real time PCR platform." 5. Section "1.0 Risk Assessment" of the IQCP under the heading "How to Reduce Error" states that the laboratory will "Train collectors on proper urine collection." The

	laboratory performs wound and fungal testing on swabs, aspirates, curettage, skin, and nails but does not test urine as a specimen source. 6. Section "1.3 Reagent" of the IQCP under the heading "How to Reduce Error" states that the laboratory will "Train testing personnel on molecular methods for upper respiratory infection." The laboratory does not perform testing for upper respiratory infections. 7. Section "1.4 Environment" of the IQCP states that a "Possible Source of Error" is "Temperatures over 27C" and "Temperatures lower than 18C." Temperature log review shows that the acceptable room temperature range for the laboratory is "20-25C." 8. Section "2.0 Quality Control Plan" of the ICQP under the heading "Control" states that the "Room temperature check and documentation" has an "Acceptance Criteria" of "18C - 27C." The acceptable room temperature range for the laboratory is "20-25C." The "Control" of "Refrigerator temperature check and documentation" has an "Acceptance Criteria" of "2C - 8C." Temperature log review shows that the acceptable refrigerator temperature range is "4-8C." 9. During an interview on 08/02/2023 at 3:50 PM, the OLC stated that the "templates" used in creating the IQCP were wrong and confirmed that the laboratory did not have an accurate IQCP in place to reduce the amount of QC required when performing RT-QPCR testing.
D5453	CONTROL PROCEDURES CFR(s): 493.1256(d)(3)(iv)(g) Unless CMS Approves a procedure, specified in Appendix C of the State Operations Manual (CMS Pub. 7), that provides equivalent quality testing, the laboratory must-- At least once a day patient specimens are assayed or examined perform the following for-- Each test system that has an extraction phase, include two control materials, including one that is capable of detecting errors in the extraction process; (g) The laboratory must document all control procedures performed. This STANDARD is not met as evidenced by: Based on review of the procedure and interview with the general supervisor (GS), the laboratory failed to run a negative extraction control for the molecular wound and fungal panel testing. Findings: 1. The laboratory ran a molecular wound testing panel and a molecular fungal testing panel. 2. The procedure for each testing panel stated that an endogenous human housekeeping gene was used as a positive extraction control and identified the well on the plate map where the control was located. The procedures did not mention a negative extraction control. 3. During the survey on 08 /02/2023 at 4:00 PM, the GS confirmed that a negative control was not run through the extraction phase of testing.
D5455	CONTROL PROCEDURES CFR(s): 493.1256(d)(3)(v)(g) Unless CMS Approves a procedure, specified in Appendix C of the State Operations Manual (CMS Pub. 7), that provides equivalent quality testing, the laboratory must-- At least once a day patient specimens are assayed or examined perform the following for-- Each molecular amplification procedure, include two control materials and, if reaction inhibition is a significant source of false negative results, a control material capable of detecting the inhibition. (g) The laboratory must document all control procedures performed. This STANDARD is not met as evidenced by:

	Based on review of the procedure and instrument data and interview with the general supervisor (GS), the laboratory failed to ensure the endogenous control (EC) was performed for the molecular fungal panel testing. Findings: 1. The GS was trained on and followed a procedure that was not the approved testing procedure (cross-refer to D5401 for findings). 2. The laboratory performed a molecular fungal panel that included an EC that amplified a human housekeeping gene to monitor any potential issues with nucleic acid extraction or inhibition of amplification. 3. The approved procedure stated that the "endogenous control must show amplification to pass" and if "all controls pass, the patient results can be reviewed and released." 4. The approved procedure stated to add patient samples into wells A-D of column 2 of the fungal panel plate where well D contained the EC. 5. At 2:00 PM on 08/02/2023, the GS confirmed that they were trained on and were following Figure 3 from the procedure located in the laboratory which showed to add patient samples into wells A-C of column 2 where well C contained the EC. As a result, the patient sample was not being added into well D of column 2 where the master mix for the EC was located and therefore could not be detected for any patient tested on the molecular fungal panel.
D5481	CONTROL PROCEDURES CFR(s): 493.1256(f)(g) (f) Results of control materials must meet the laboratory's and, as applicable, the manufacturer's test system criteria for acceptability before reporting patient test results. (g) The laboratory must document all control procedures performed. This STANDARD is not met as evidenced by: Based on record review and interview with the outside laboratory consultant (OLC), the laboratory failed to document all activities completed for the risk assessment (RA) in the laboratory's Individualized Quality Control Plan (IQCP), including data to support their RA decisions. Findings: 1. The laboratory prepared an IQCP to reduce the amount of quality control performed for molecular wound and fungal panel testing. 2. The laboratory is required to perform a RA as part of the IQCP, which identifies, evaluates, and documents all potential failures and errors in the entire testing process (preanalytic, analytic, and postanalytic phases) and must include, at minimum, an evaluation of the following five components: Specimen, Test system, Reagent, Environment, and Testing personnel. 3. A review of the laboratory's IQCP showed that the RA included a summary of the potential errors associated with the required five components, but did not include a copy of the data which was reviewed to establish the IQCP. 4. During an interview on 08/02/2023 at 3:50 PM, the OLC confirmed that the laboratory did not maintain a copy of all documents reviewed while performing the RA.
D5805	TEST REPORT CFR(s): 493.1291(c) The test report must indicate the following: (c)(1) For positive patient identification, either the patient's name and identification number, or a unique patient identifier and identification number. (c)(2) The name and address of the laboratory location where the test was performed. (c)(3) The test report date. (c)(4) The test performed. (c)(5) Specimen source, when appropriate. (c)(6) The test result and, if applicable, the units of measurement or interpretation, or both. (c)(7) Any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability.

	This STANDARD is not met as evidenced by: Based on review of final patient reports and interview with the outside laboratory consultant (OLC), the laboratory failed to ensure that the information provided on the final test report was accurate. Findings: 1. A random review of final patient reports showed that the name of the laboratory where testing is performed is listed as "Annapolis Foot & Ankle Center." This does not match the name of the laboratory on the CLIA license, which is "John G Deleonibus DPM PA." 2. The first page of the "Molecular PCR [polymerase chain reaction] Lab Report" lists the results of the laboratory's fungal PCR testing under the heading, "Test Performed" as "Vaginitis Pathogens." The specimen "source" on the final report states that the test was performed on "nail clipping." 3. During an interview on 08/02/2023 at 3:23 PM, the OLC confirmed that the information provided on the final test report was not accurate.
D6076	LABORATORY DIRECTOR CFR(s): 493.1441 The laboratory must have a director who meets the qualification requirements of 493. 1443 of this subpart and provides overall management and direction in accordance with 493.1445 of this subpart. This CONDITION is not met as evidenced by: Based on record review and interview with the general supervisor (GS) and the outside laboratory consultant, the lab director failed to update the delegation of responsibilities to the new technical supervisor (TS) (D6079); failed to ensure that stability and validation studies for the molecular wound panel assay were performed with the correct specimen matrix and that the validation study summaries for the molecular wound panel and fungal panel assays described how all performance specifications were determined (D6086); failed to ensure that proficiency testing results were reviewed and evaluated (D6091); failed to ensure that an investigation was performed and corrective actions taken when the laboratory did not receive acceptable results for alternative split sample proficiency testing (D6092); failed to establish an Individualized Quality Control Plan (IQCP) based on accurate data and failed to maintain documentation of all activities completed for the risk assessment in the laboratory's IQCP (D6093); failed to ensure that there was a quality assessment program in place capable of identifying errors in the test system to ensure accuracy and reliability of patient test results (D6094); failed to ensure that the TS received the appropriate training to perform their duties as TS (D6102); and failed to ensure that the GS was trained on and had access to the approved procedure for molecular fungal panel testing (D6106).
D6079	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(a)(b) The laboratory director is responsible for the overall operation and administration of the laboratory, including the employment of personnel who are competent to perform test procedures, record and report test results promptly, accurately and proficiently, and for assuring compliance with the applicable regulations. (a) The laboratory director, if qualified, may perform the duties of the technical supervisor, clinical consultant, general supervisor, and testing personnel, or delegate these responsibilities to personnel meeting the qualifications under 493.1447, 493.1453, 493.1459, and

	493.1487 respectively. (b) If the laboratory director reapportions performance of his or her responsibilities, he or she remains responsible for ensuring that all duties are properly performed. This STANDARD is not met as evidenced by: Based on procedure manual review and interview with the general supervisor (GS), the lab director (LD) failed to update the delegation of responsibilities to the new technical supervisor (TS). Findings: 1. The procedure, "Laboratory Director Delegation of Responsibilities" provides a list of duties which the LD delegated to the TS. The procedure was signed by the LD and the former TS (who is not listed on the "Laboratory Personnel Report" [CMS-209]) on 07/25/2022. 2. During an interview on 08/02/2023 at 9:40 AM the GS stated that the new TS listed on the CMS-209 started in March, 2023 and confirmed that the LD did not update the delegation of responsibilities to the new TS.
D6086	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(e)(3)(ii) The laboratory director must ensure that verification procedures used are adequate to determine the accuracy, precision, and other pertinent performance characteristics of the method. This STANDARD is not met as evidenced by: The laboratory director failed to ensure that stability and validation studies for the molecular wound panel assay were performed with the correct specimen matrix and that the validation study summaries for the molecular wound panel and fungal panel assays described how all performance specifications were determined. Cross-refer to tags D5311 and D5423 for findings. 43123
D6091	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(e)(4)(iii) The laboratory director must ensure all proficiency testing reports received are reviewed by the appropriate staff to evaluate the laboratory's performance and to identify any problems that require corrective action. This STANDARD is not met as evidenced by: The laboratory director failed to ensure that proficiency testing results were reviewed and evaluated. Cross-refer to tags D5211 and D5215 for findings.
D6092	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(e)(4)(iv) The laboratory director must ensure an approved corrective action plan is followed when any proficiency testing result is found to be unacceptable or unsatisfactory. This STANDARD is not met as evidenced by:

	The laboratory director failed to ensure that an investigation was performed and corrective actions taken when the laboratory did not receive acceptable results for alternative split sample proficiency testing. Cross-refer to tag D5211 for findings.
D6093	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(e)(5) The laboratory director must ensure that the quality control programs are established and maintained to assure the quality of laboratory services provided and to identify failures in quality as they occur. This STANDARD is not met as evidenced by: The laboratory director failed to establish an Individualized Quality Control Plan (IQCP) based on accurate data and failed to maintain documentation of all activities completed for the risk assessment in the laboratory's IQCP. Cross-refer to D5445 and D5481 for findings.
D6094	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(e)(5) The laboratory director must ensure that the quality assessment programs are established and maintained to assure the quality of laboratory services provided and to identify failures in quality as they occur. This STANDARD is not met as evidenced by: Based on review of the procedure, validation summaries, and final reports and interview with the outside laboratory consultant (OLC), the laboratory director did not ensure that there was a quality assessment (QA) program in place capable of identifying errors in the test system to ensure accuracy and reliability of patient test results. Findings: 1. The laboratory reported patient results for the molecular wound and fungal panel assays when specimens were received and tested beyond the determined stability limits. Cross-refer to tag D5311 for findings. 2. The laboratory reported patient results for the molecular fungal panel assay when the endogenous control (EC) was not tested. Cross-refer to tag D5455 for findings. 3. The OLC confirmed that patient results were reported when specimens were tested outside their determined stability and when the EC was not tested on the fungal panel. The laboratory QA program was not able to identify these failures in quality.
D6102	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(e)(12) The laboratory director must ensure that prior to testing patients' specimens, all personnel have the appropriate education and experience, receive the appropriate training for the type and complexity of the services offered, and have demonstrated that they can perform all testing operations reliably to provide and report accurate results. This STANDARD is not met as evidenced by: Based on record review and interview with the general supervisor (GS), the laboratory director (LD) failed to ensure that the technical supervisor (TS) received the

	appropriate training to perform their duties as TS. Findings: 1. Record review showed that the initial training for the TS was performed on 05/12/2023, however the GS stated that the TS had been reviewing quality control and quality assurance records since March, 2023. 2. During an interview on 08/02/2023 at 3:50 PM, the GS confirmed that the LD did not ensure that the TS was trained before they began performing their duties as a TS.
D6106	LABORATORY DIRECTOR RESPONSIBILITIES CFR(s): 493.1445(e)(14) The laboratory director must ensure that an approved procedure manual is available to all personnel responsible for any aspect of the testing process. This STANDARD is not met as evidenced by: The laboratory director failed to ensure that the general supervisor was trained on and had access to the approved procedure for molecular fungal panel testing. Cross-refer to tags D5401 and D5455 for findings.
D6108	LABORATORY TECHNICAL SUPERVISOR CFR(s): 493.1447 The laboratory must have a technical supervisor who meets the qualification requirements of 493.1449 of this subpart and provides technical supervision in accordance with 493.1451 of this subpart. This CONDITION is not met as evidenced by: Based on record review and interview with the general supervisor and the outside laboratory consultant, the technical supervisor failed to ensure that stability and validation studies for the molecular wound panel assay were performed with the correct specimen matrix and that the validation study summaries for the molecular wound panel and fungal panel assays described how all performance specifications were determined and included a discussion of unexpected results (D6115), and failed to ensure that a negative extraction control was tested for the molecular wound and fungal panel assays and the endogenous control was run for the molecular fungal panel assay (D6117).
D6115	TECHNICAL SUPERVISOR RESPONSIBILITIES CFR(s): 493.1451(b)(2) The technical supervisor is responsible for verification of the test procedures performed and establishment of the laboratory's test performance characteristics, including the precision and accuracy of each test and test system. This STANDARD is not met as evidenced by: The technical supervisor failed to ensure that stability and validation studies for the molecular wound panel assay were performed with the correct specimen matrix and that the validation study summaries for the molecular wound panel and fungal panel assays described how all performance specifications were determined and included a discussion of unexpected results. Cross-refer to tags D5311 and D5423 for details.

D6117	TECHNICAL SUPERVISOR RESPONSIBILITIES CFR(s): 493.1451(b)(4) The technical supervisor is responsible for establishing a quality control program appropriate for the testing performed and establishing the parameters for acceptable levels of analytic performance and ensuring that these levels are maintained throughout the entire testing process from the initial receipt of the specimen, through sample analysis and reporting of test results. This STANDARD is not met as evidenced by: The technical supervisor failed to ensure that a negative extraction control was tested for the molecular wound and fungal panel assays and the endogenous control was run for the molecular fungal panel assay. Cross-refer to tags D5453 and D5455 for findings.