510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K243544

B Applicant

BioFire Diagnostics, LLC

C Proprietary and Established Names

BIOFIRE SPOTFIRE Respiratory/Sore Throat Panel Mini

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-CoV-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to add the Anterior Nasal Swab (ANS) specimen type to the previously cleared BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini and demonstrate its substantial equivalence.

B Measurand:

The respiratory menu of the BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini detects severe acute respiratory syndrome (SARS-CoV-2), influenza A, influenza B, respiratory syncytial virus (RSV), and human rhinovirus RNA from nasopharyngeal and anterior nasal swab specimens in collection medium from patients with signs and symptoms of respiratory infection.

The sore throat menu of the BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini detects RNA from SARS-CoV-2, influenza A, influenza B, respiratory syncytial virus (RSV) viruses and DNA from *Streptococcus pyogenes* (Group A *Streptococcus*) in throat swabs in collection medium.

C Type of Test:
Multiplex nucleic acid assay for use with the BIOFIRE SPOTFIRE System for the qualitative detection and identification of viral and/or bacterial pathogens in nasopharyngeal or anterior nasal swabs from patients with signs and symptoms of respiratory tract infection (BIOFIRE SPOTFIRE R Panel) or in throat swabs from patients with signs and symptoms of pharyngitis (BIOFIRE SPOTFIRE ST Panel).

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini is an automated multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE SPOTFIRE System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) or anterior nasal swab (ANS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19 (Respiratory menu); or in throat swab (TS) specimens from individuals with signs and symptoms of pharyngitis (Sore Throat menu).

The following analytes are identified and differentiated using the BIOFIRE SPOTFIRE R/ST Panel Mini:

Respiratory Menu:
Viruses
Coronavirus SARS-CoV-2
Human rhinovirus
Influenza A virus
Influenza B virus
Respiratory syncytial virus

Sore Throat Menu:
Viruses
Human rhinovirus
Influenza A virus
Influenza B virus
Respiratory syncytial virus

Bacteria
Streptococcus pyogenes (group A Strep)

Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS/ANS/TS specimens during the acute phase of infection. The detection and

identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection and/or pharyngitis is indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patientmanagement decisions.

Negative results in the setting of a respiratory illness and/or pharyngitis may be due to infection with pathogens that are not detected by this test, or a respiratory tract infection that may not be detected by a NPS, ANS, or TS specimen. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the BIOFIRE SPOTFIRE R/ST Panel Mini may not be the definite cause of disease.

Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) maybe necessary when evaluating a patient with possible respiratory tract infection and/or pharyngitis.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only
For *in vitro* diagnostic Use Only

D Special Instrument Requirements:
For use with the BIOFIRE SPOTFIRE System, only.

IV Device/System Characteristics:

A Device Description:
The BIOFIRE SPOTFIRE R/ST Panel Mini simultaneously identifies five (5) different respiratory viral pathogens in nasopharyngeal swabs (NPS) or anterior nasal swabs (ANS), or five (5) different viral and bacterial pharyngitis pathogens in throat swabs (TS) from individuals with signs and symptoms of respiratory tract infections or pharyngitis, respectively. The BIOFIRE SPOTFIRE R/ST Panel Mini is compatible with the BIOFIRE SPOTFIRE System, a polymerase chain reaction (PCR)-based *in vitro* diagnostic system for infectious disease testing. The BIOFIRE SPOTFIRE System Software executes the BIOFIRE SPOTFIRE R/ST Panel Mini test and interprets and reports the test results.

B Principle of Operation:
A test is initiated by loading Hydration Solution into the hydration solution injection port of the BIOFIRE SPOTFIRE R/ST Panel Mini pouch and NPS, ANS, or TS specimen, mixed with the provided Sample Buffer, into the other sample injection port of the BIOFIRE SPOTFIRE R/ST Panel Mini pouch and placing it in the BIOFIRE SPOTFIRE System. The pouch contains all the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the BIOFIRE SPOTFIRE System Software guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run.

Nucleic acid extraction occurs within the BIOFIRE SPOTFIRE R/ST Panel Mini pouch using mechanical and chemical lysis followed by purification using magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, the BIOFIRE SPOTFIRE

System performs a nested multiplex PCR that is executed in two stages. During the first stage, the BIOFIRE SPOTFIRE System performs a single, large volume, highly multiplexed reverse transcription PCR (rt-PCR) reaction. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double- stranded DNA binding dye. The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, is performed in singleplex fashion in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the 2nd stage PCR captures fluorescent images of the PCR reactions and software interprets the data.

The BIOFIRE SPOTFIRE System Software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the BIOFIRE SPOTFIRE R/ST Panel Mini.

C Instrument Description Information:

1. Instrument Name:
   BIOFIRE SPOTFIRE System

2. Specimen Identification:
   Specimen identification is entered via barcode.

3. Specimen Sample and Handling:
   Nasopharyngeal Swab (NPS) or Anterior Nasal Swab (ANS) in viral transport medium, or Throat Swab (TS) specimens in liquid Amies Medium.

4. Calibration
   The BIOFIRE SPOTFIRE System is factory calibrated. User calibration is not required.

5. Quality Control:
   Please refer to the Quality Control information previously reviewed and presented in the K232954 FDA Decision Summary.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini

B Predicate 510(k) Number(s):
K241194

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243544	K241194
Device Trade Name	Same	BIOFIRE SPOTFIRE

		Respiratory/Sore Throat (R/ST) Panel Mini
Regulation	Same	21 CFR 866.3981
Product Code	Same	QOF
Prescription Use Only	Same	Yes
General Device Characteristic Similarities		
Intended Use/Indications For Use	The BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini is an automated, multiplexed, polymerase chain reaction (PCR) test intended for use with the BIOFIRE SPOTFIRE System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19 (Respiratory menu); or in throat swab (TS) specimens from individuals with signs and symptoms of pharyngitis (Sore Throat menu). The following analytes are identified and differentiated using the BIOFIRE SPOTFIRE R/ST Panel Mini: Respiratory Menu Viruses: Coronavirus SARS-CoV-2, Human rhinovirus, Influenza A virus, Influenza B virus,	The BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE SPOTFIRE System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19; (Respiratory menu) or in throat swab (TS) specimens from individuals with signs and symptoms of pharyngitis (Sore Throat menu). The following analytes are identified and differentiated using the SPOTFIRE R/ST Panel Mini: Respiratory Menu Viruses: Coronavirus SARS-CoV-2, Human rhinovirus, Influenza A, Influenza B, Respiratory syncytial virus (RSV)

	Respiratory syncytial virus Sore Throat Menu: Viruses Human rhinovirus Influenza A virus Influenza B virus Respiratory syncytial virus Bacteria *Streptococcus pyogenes* (group A Strep) Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS/ANS/TS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection and/or pharyngitis is indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness and/or pharyngitis may be due to infection	Sore Throat Menu: Human rhinovirus, Influenza A, Influenza B, Respiratory syncytial virus (RSV) *Streptococcus pyogenes* (group A Strep). Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS/TS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection and/or pharyngitis is indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness and/or pharyngitis may be due to infection with pathogens that are not detected by this test, or a respiratory tract infection that may not be detected by an NPS or TS specimen. Positive results do not rule out co-infection with other organisms. The agent(s)

	with pathogens that are not detected by this test, or a respiratory tract infection that may not be detected by a NPS, ANS, or TS specimen. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the BIOFIRE SPOTFIRE R/ST Panel Mini may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection and/or pharyngitis.	detected by the SPOTFIRE R/ST Panel Mini may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection and/or pharyngitis.
Intended User	Same	Professional Use
Principle of Operation	Same	Highly multiplexed nested nucleic acid amplification test with melt analysis
Analytes	Same	RNA/DNA
Organisms Detected	Same	<u>Respiratory Menu</u> Viruses Coronavirus SARS-CoV-2 Human rhinovirus Influenza A virus Influenza B virus Respiratory syncytial virus <u>Sore Throat Menu</u> Human rhinovirus Influenza A virus Influenza B virus Respiratory syncytial virus *Streptococcus pyogenes*

		(group A Strep)
Instrumentation	Same	BIOFIRE SPOTFIRE System
Time to Result	Same	~15 minutes
Reagent Storage	Same	Room Temperature
Test Interpretation	Same	Automated test interpretation and reporting. User cannot access raw data.
Intended User	Same	Professional Use
General Device Characteristic Differences		
Specimen Type	Nasopharyngeal swab in transport medium or Anterior nasal swab in transport medium or Throat swab in transport medium	Nasopharyngeal swab in transport medium or Throat swab in transport medium

VI Standards/Guidance Documents Referenced:

- Class II Special Controls as per 21 CFR 866.3981

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   a. Within-Laboratory Precision
   Please refer to the Within-Laboratory Precision Study data previously reviewed and presented in the K232954 FDA Decision Summary.

   b. Reproducibility
   Please refer to the Reproducibility Study data previously reviewed and presented in the K232954 FDA Decision Summary.

2. Linearity:
   Not applicable; this is a qualitative assay.

3. Analytical Specificity/Interference:

Analytical Reactivity (Inclusivity)

a. Wet-Testing

Please refer to the Inclusivity data previously reviewed and presented in the K232954 FDA Decision Summary.

b. In silico

Please refer to the *In Silico* Study data previously reviewed and presented in the K232954 FDA Decision Summary.

Cross-Reactivity (Exclusivity)

Cross-reactivity (exclusivity) of the BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini was evaluated by testing on-panel and off-panel microorganisms. The on-panel exclusivity evaluation assessed the potential for intra-panel cross-reactivity by testing representative organisms that are targeted by the panel. The off-panel exclusivity evaluation assessed the potential for cross-reactivity of panel assays with organisms not detected by the panel. The organisms evaluated included relevant bacteria, fungi, and viruses that are either phylogenetically related to organisms detected by the BIOFIRE SPOTFIRE R/ST Panel Mini or pathogenic/commensal organisms that could potentially be present in NPS, ANS or TS specimens. Each organism was tested in triplicate at the highest possible concentration (generally ≥1.0E+07 units/mL for bacteria and ≥1.0E+05 units/mL for viruses) in the absence of target analyte. The results and the concentrations tested are presented in **Table 1** and **Table 2.**

Table 1: Summary of Results for Cross-Reactivity Testing with On-Panel Organisms

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
SARS-CoV-2[a]	ATCC VR-1986HK	7.6E+07 copies/mL	None
Enterovirus D68	ATCC VR-1823	1.6E+07 $TCID_{50}$/mL	None
Human rhinovirus A1	ZeptoMetrix 0810012CFN	1.3E+06 $TCID_{50}$/mL	None
Influenza A H1N1pdm09	ZeptoMetrix 0810538CF	1.4E+05 $TCID_{50}$/mL	None
Influenza A H3N2	ZeptoMetrix 0810526CF	7.2E+05 $TCID_{50}$/mL	None
Influenza B (Victoria Lineage)	BEI NR-44023	2.8E+08 $CEID_{50}$/mL	None
	ZeptoMetrix 0810037CF	2.5E+05 $TCID_{50}$/mL	None
Influenza B (Yamagata Lineage)	ZeptoMetrix 0810256CF	2.1E+04 $TCID_{50}$/mL	None
Respiratory syncytial virus A	ZeptoMetrix 0810040ACF	4.2E+05 $TCID_{50}$/mL	None
Respiratory syncytial virus B	ZeptoMetrix 0810479CF	4.2E+05 $TCID_{50}$/mL	None
Streptococcus pyogenes	ATCC 12344	3.4E+08 cells/mL	None

[a] Heat-inactivated

Table 2: Summary of Results for Cross-Reactivity Testing with Off-Panel Organisms

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Bacteria			
Arcanobacterium bernardiae	ATCC BAA-441	1.6E+09 cells/mL	None
Arcanobacterium haemolyticum	ATCC 9345	1.5E+08 cells/mL	None
Arcanobacterium pyogenes	ATCC 49698	6.7E+09 cells/mL	None
Bacillus cereus	ATCC 7064	8.3E+09 cells/mL	None
Bordetella bronchiseptica	ATCC 10580	8.3E+09 cells/mL	None
	ATCC 4617	7.9E+09 cells/mL	None
	ATCC 19395	7.9E+09 cells/mL	None
	NRRL B-59914	7.1E+09 cells/mL	None
Bordetella holmesii	ATCC 700052	8.3E+09 cells/mL	None
Bordetella parapertussis	ZeptoMetrix 0801462	4.6E+09 CFU/mL	None
Bordetella pertussis	ZeptoMetrix 0801459	1.3E+10 CFU/mL	**Human rhinovirus**[a]
		1.3E+09 CFU/mL	None
Burkholderia cepacia	ATCC 51671	1.3E+09 CFU/mL	None
Campylobacter rectus	ATCC 33238	7.6E+07 cells/mL	None
Chlamydia pneumoniae	ATCC 53592	2.9E+07 IFU/mL	None
Chlamydia trachomatis	ZeptoMetrix 0801775	1.3E+08 IFU/mL	None
Corynebacterium diptheriae	ATCC 27010	8.0E+09 cells/mL	None
Corynebacterium pseudodiphtheriticum	ATCC 10700	8.7E+09 cells/mL	None
Eikenella corrodens	ATCC BAA-1152	8.2E+09 cells/mL	None
Enterococcus casseliflavus	ATCC 49605	8.0E+09 cells/mL	None
Enterococcus faecalis	ZeptoMetrix 0801637	8.0E+08 CFU/mL	None
Escherichia coli	ATCC BAA-2196	7.2E+09 cells/mL	None
Fusobacterium necrophorum ssp. *funduliforme*	ATCC 51357	4.4E+08 cells/mL	None
Fusobacterium nucleatum	ATCC 25586	4.9E+08 cells/mL	None
Fusobacterium varium	ATCC 27725	1.6E+08 cells/mL	None
Gemella haemolysans	ATCC 10379	4.0E+09 cells/mL	None
Gemella morbillorum	ATCC 27824	1.0E+08 cells/mL	None
Granulicatella adiacens	ATCC 49175	1.3E+09 cells/mL	None
Haemophilus influenzae	ATCC 10211	8.3E+09 cells/mL	None
Haemophilus parahaemolyticus	ATCC 49700	8.7E+09 cells/mL	None
Klebsiella pneumoniae	CDC AR#0115	7.3E+09 CFU/mL	None
Lactobacillus rhamnosus	ATCC 7469	7.9E+09 cells/mL	None
Lactococcus lactis	ATCC 29146	6.2E+09 cells/mL	None
Legionella pneumophila	ATCC 33215	7.0E+09 cells/mL	None
Leptotrichia buccalis	ATCC 14201	4.4E+08 cells/mL	None
Moraxella catarrhalis	ATCC 43627	7.2E+09 cells/mL	None
Mycobacterium tuberculosis	ZeptoMetrix 0801660	6.1E+06 CFU/mL	None
Mycoplasma buccale	Mycoplasma Experience NC10136	1.4E+07 CFU/mL	None
Mycoplasma faucium	Mycoplasma Experience NC10174	1.4E+06 CFU/mL	None
Mycoplasma fermentans	Mycoplasma Experience NC10117	2.8E+07 CFU/mL	None
Mycoplasma genitalium	Mycoplasma Experience NC10195	1.8E+06 CFU/mL	None

Mycoplasma hominis	Mycoplasma Experience NC10111	1.2E+07 CFU/ml	None
Mycoplasma lipophilum	Mycoplasma Experience NC10173	1.5E+06 CFU/mL	None
Mycoplasma orale	Mycoplasma Experience NC10112	2.2E+07 CFU/mL	None
Mycoplasma pneumoniae	ZeptoMetrix 0801579	2.5E+07 CCU/mL	None
Mycoplasma salivarium	Mycoplasma Experience NC10113	4.4E+06 CFU/mL	None
Neisseria elongata	ATCC 25295	8.5E+09 cells/mL	None
Neisseria gonorrhoeae	ZeptoMetrix 0801482	4.9E+07 CFU/mL	None
Neisseria lactamica	ATCC 23971	2.7E+09 cells/mL	None
Neisseria meningitidis	ATCC 13113	7.4E+09 cells/mL	None
Mycoplasma pneumoniae	ZeptoMetrix 0801579	2.5E+07 CCU/mL	None
Neisseria sicca	ATCC 9913	7.2E+09 cells/mL	None
Neisseria subflava	ATCC 49275	8.0E+09 cells/mL	None
Nocardia brasiliensis	ATCC 19019	1.2E+09 CFU/mL	None
Parvimonas micra[b]	ATCC 33270	6.0E+07 cells/mL	None
Pneumocystis carinii	ATCC PRA-159	1.0E+07 nuclei/mL	None
Porphyromonas endodontalis	ATCC 35406	1.6E+07 cells/mL	None
Porphyromonas gingivalis	ATCC BAA-308	5.0E+08 cells/mL	None
Prevotella histicola	BEI HM-471	9.0E+08 cell/mL	None
Prevotella melaninogenica	ATCC 25845	6.9E+08 cells/mL	None
Prevotella oralis	ATCC 33322	6.2E+08 cells/mL	None
Pseudomonas aeruginosa	CDC AR#0092	8.3E+09 cells/mL	None
Rhodococcus equi	ATCC 33706	7.3E+09 cells/mL	None
Serratia marcescens	ATCC 27137	8.9E+09 cells/mL	None
Staphylococcus aureus	ATCC BAA-1700	7.4E+09 cells/mL	None
Staphylococcus epidermidis	ATCC 12228	8.0E+09 cells/mL	None
Staphylococcus haemolyticus	ATCC 29968	8.0E+09 cells/mL	None
Staphylococcus intermedius	ATCC 29663	8.2E+09 cells/mL	None
Staphylococcus saprophyticus	ATCC 15305	8.1E+09 cells/mL	None
Streptococcus agalactiae	ATCC 13813	6.0E+09 cells/mL	None
Streptococcus anginosus	ATCC 700231	7.1E+09 cells/mL	None
Streptococcus constellatus ssp. pharyngis	NCTC 13122	5.6E+08 cells/mL	None
Streptococcus dysgalactiae ssp. dysgalactiae	ATCC 43078	6.7E+09 cells/mL	None
	NCTC 4669	7.4E+09 cells/mL	None
	NCTC 4335	8.4E+09 cells/mL	None
	NCTC 4670	6.6E+09 cells/mL	None
	CCUG 27665	7.4E+09 cells/mL	None
	CCUG 28112	6.7E+09 cells/mL	None
	CCUG 28114	7.5E+09 cells/mL	None
Streptococcus dysgalactiae ssp. Equisimilis (Isolated from human)	ZeptoMetrix 0801516	7.8E+08 CFU/mL	None
Streptococcus dysgalactiae ssp. Equisimilis (Isolated from pig)	CCUG 28117	7.1E+09 cells/mL	None
Streptococcus dysgalactiae ssp. Equisimilis (Isolated from horse)	CCUG 27664	7.5E+09 cells/mL	None
	ATCC 10009	6.9E+09 cells/mL	None
Streptococcus gallolyticus	ATCC 43143	2.8E+09 cells/mL	None
Streptococcus gordonii	ATCC 10558	4.5E+09 cells/mL	None

Streptococcus intermedius	ATCC 27335	2.9E+09 cells/mL	None
Streptococcus mitis	ATCC 15914	3.2E+09 cells/mL	None
Streptococcus mutans	ATCC 25175	2.3E+09 cells/mL	None
Streptococcus oralis	ATCC 10557	1.1E+09 cells/mL	None
Streptococcus parasanguinis	ATCC 15912	7.8E+09 cells/mL	None
Streptococcus pneumoniae	ATCC 49619	2.5E+08 cells/mL	None
Streptococcus salivarius	ATCC 13419	6.6E+09 cells/mL	None
Streptococcus sanguinis	ATCC 10556	1.1E+09 cells/mL	None
Tannerella forsythia	ATCC BAA-2717	2.6E+08 cells/mL	None
Treponema denticola	ATCC 33520	2.2E+08 cells/mL	None
Ureaplasma urealyticum	ATCC 27618	5.7E+07 cells/mL	None
Veillonella parvula	ATCC 10790	4.7E+08 cells/mL	None
Fungi			
Candida albicans	ATCC MYA-2876	2.8E+08 cells/mL	None
Pneumocystis jirovecii	ATCC PRA-159	2.0E+07 nuclei/mL	None
Saccharomyces cerevisiae	ATCC 18824	1.9E+08 cells/mL	None
Viruses			
Adenovirus A	ZeptoMetrix 0810073CF	1.4E+05 $TCID_{50}$/mL	None
Adenovirus B	ZeptoMetrix 0810062CF	1.2E+07 $TCID_{50}$/mL	None
Adenovirus C	ZeptoMetrix 0810110CF	2.2E+06 $TCID_{50}$/mL	None
Adenovirus D	ZeptoMetrix 0810119CF	1.7E+05 $TCID_{50}$/mL	None
Adenovirus E	ZeptoMetrix 0810070CF	1.4E+05 $TCID_{50}$/mL	None
Adenovirus F	ZeptoMetrix 0810085CF	1.1E+06 $TCID_{50}$/mL	None
Human Coronavirus 229E	ATCC VR-740	8.9E+06 $TCID_{50}$/mL	None
Human Coronavirus HKU1	Clinical Specimens	4.5E+07 copies/mL	None
Human Coronavirus NL63	ZeptoMetrix 0810228CF	5.0E+05 $TCID_{50}$/mL	None
Human Coronavirus OC43	ZeptoMetrix 0810024CF	3.6E+05 $TCID_{50}$/mL	None
Cytomegalovirus	ZeptoMetrix 0810003CF	1.9E+05 $TCID_{50}$/mL	None
Epstein-Barr virus	ZeptoMetrix 0810008CF	5.9E+06 copies/mL	None
Human herpes simplex virus 1	ATCC VR-260	8.9E+06 $TCID_{50}$/mL	None
Human metapneumovirus A1	ZeptoMetrix 0810161CF	2.5E+05 $TCID_{50}$/mL	None
Human metapneumovirus A2	ZeptoMetrix 0810164CF	3.6E+05 $TCID_{50}$/mL	None
Human metapneumovirus B1	ZeptoMetrix 0810156CF	1.6E+04 $TCID_{50}$/mL	None
Human metapneumovirus B2	ZeptoMetrix 0810162CF	1.3E+06 $TCID_{50}$/mL	None
Measles virus	ZeptoMetrix 0810025CF	2.5E+05 $TCID_{50}$/mL	None
Middle East respiratory syndrome coronavirus (heat-inactivated)	ZeptoMetrix 0810575CFHI	1.2E+05 $TCID_{50}$/mL	None
Mumps virus	ZeptoMetrix 0810079CF	2.0E+06 $TCID_{50}$/mL	None
Parainfluenza virus 1	ZeptoMetrix 0810014CF	4.2E+05 $TCID_{50}$/mL	None
Parainfluenza virus 2	ZeptoMetrix 0810015CF	1.2E+07 $TCID_{50}$/mL	None
Parainfluenza virus 3	ZeptoMetrix 0810016CF	3.4E+07 $TCID_{50}$/mL	None
Parainfluenza virus 4	ZeptoMetrix 0810060CF	3.4E+07 $TCID_{50}$/mL	None
Severe acute respiratory syndrome coronavirus (purified genomic RNA)	BEI NR-52346	5.3E+05 genomes/mL	None

[a] The Human rhinovirus assay may amplify off-target sequences found in strains of *Bordetella* species (*B. pertussis, B. parapertussis,* and *B. bronchiseptica*) when present at a concentration ≥1.3E+10 CFU/mL.

[b] Parvimonas micra was formerly classified as Micromonas micros and Peptostreptococcus micro.

Competitive Interference Study

This study evaluated potential competitive interference between analytes on the BIOFIRE SPOTFIRE R/ST Panel Mini. Testing evaluated the potential for competition or interference from the microorganism present at a high concentration to affect detection of the analytes near their Limit of Detection (LoD). The high-level microorganisms tested include common microbiota and pathogens of the upper respiratory tract. Contrived co-spiked samples containing representative organisms ware prepared in an artificial throat swab matrix at 3x the LoD. The panel composition is presented in **Table 3**. Testing was performed by spiking a high concentration of a potentially competing microorganism into the contrived co-spiked pooled sample. Negative sample matrix was also spiked with the test organisms as the negative control. Each panel was tested in replicates of three. All samples gave the expected positive and negative results at the concentrations tested. The concentration of each microorganism tested and results from study are summarized in **Table 4**.

In a separate study (refer to Sections VII (A)(6) and (B)(2), in K232954 Decision Summary) the analytical sensitivity of the BIOFIRE SPOTFIRE R/ST Panel was shown to be similar, irrespective of the specimen matrix (nasopharyngeal or throat swab) and therefore it was considered acceptable to use just one of the two matrices for the evaluation of assay interference.

Table 3: Representative On-Panel Analytes Used to Evaluate Assay Interference.

Analyte[a]	Organism Type	Source ID	Test Concentration (3x LoD)
Influenza B Victoria Florida/02/06	Enveloped, negative sense, single-stranded RNA virus	ZeptoMetrix 0810037CF	9.9E-02 $TCID_{50}$/mL
Human rhinovirus A1	Non-enveloped, positive sense, single-stranded RNA virus	ZeptoMetrix 0810012CFN	6.3E-01 $TCID_{50}$/mL
Streptococcus pyogenes SF-120 T-type 1	gram-positive bacterium	ATCC 12344	1.4E+03 cells/mL

IFU: Inclusion Forming Units; $TCID_{50}$: Tissue Culture Infectious Dose-50%

[a] Testing was performed using the released IVD BIOFIRE SPOTFIRE Respiratory (R) Panel, which is identical in chemical composition to the BIOFIRE R/ST Panel Pro used in the remainder of the testing. The data produced was reanalyzed with the Respiratory/Sore Throat Panel v1.1 Panel Software v4.0.1 to provide a consistent analysis for all testing. The BIOFIRE SPOTFIRE R/ST Panel Mini does not include Adenovirus, Human Coronavirus NL63, or *Chlamydia pneumoniae*. Panel analytes were representative of all analytes evaluated by the BIOFIRE SPOTFIRE R/ST Panel Pro.

Table 4: Summary of Organisms and Concentrations Tested for Competitive Interference

Organism Tested	Concentration Tested	Observed Interference
On-Panel		
Enterovirus D68	7.8 E+07 copies/mL	None
Respiratory syncytial virus A	1.5E+07 copies/mL	None
Streptococcus pyogenes	2.2E+08 copies/mL	None
Off-Panel		
Adenovirus A31	1.6E+07 copies/mL	None
Coronavirus 229E	1.5E+07 copies/mL	None
Cytomegalovirus (CMV)	4.2E+04 $TCID_{50}$/mL	None
Epstein-Barr virus (EBV)	5.9E+06 copies/mL	None
Herpes simplex virus 1	9.0E+06 $TCID_{50}$/mL	None
Parainfluenza virus 3	8.0E+06 copies/mL	None

Bordetella pertussis	1.6E+09 copies/mL	None
Eikenella corrodens	8.2E+08 Cells/mL	None
Haemophilus influenzae	8.3E+08 CFU/mL	None
Lactobacillus rhamnosus	7.9E+08 Cells/mL	None
Legionella pneumophila	7.0E+08 Cells/mL	None
Neisseria meningitidis	7.4E+08 Cells/mL	None
Nocardia brasiliensis	1.2E+08 CFU/mL	None
Porphyromonas gingivalis	5.0E+07 Cells/mL	None
Prevotella oralis	6.2E+07 Cells/mL	None
Pseudomonas aeruginosa	8.3E+08 Cells/mL	None
Staphylococcus aureus	7.4E+08 CFU/mL	None
Staphylococcus epidermidis	8.0E+08 Cells/mL	None
Streptococcus mitis	3.2E+08 Cells/mL	None
Streptococcus mutans	2.3E+08 Cells/mL	None
Streptococcus pneumoniae	2.5E+07 CFU/mL	None
Streptococcus salivarius	6.6E+08 Cells/mL	None
Candida albicans	2.8E+07 CFU/mL	None

Interfering Substances

To evaluate the potential for various endogenous and exogenous substances that may be present in patient's samples to interfere with the performance of the BIOFIRE SPOTFIRE R/ST Panel Mini, contrived co-spiked samples containing representative organisms were prepared in an artificial throat swab matrix at 3x LoD. The panel composition is presented in **Table 3** above. Positive and negative samples were prepared by adding potential interfering substances to pools containing the representative organisms (for positive samples) or to organism-free pools (for negative samples). The samples were tested in triplicate.

False negative results were reported for human rhinovirus/enterovirus in the presence of nasal spray, Ibuprofen, and Snuff (Tobacco). Two additional replicates were tested and gave the expected results. The results from the study and concentrations of the potentially interfering substances tested are presented in **Table 5-7**.

Table 5: BIOFIRE SPOTFIRE R/ST Panel Mini Results for Endogenous Substance Interference Testing

Substance Tested Concentration Tested	**Sample**	**Positive Results Reported/Replicates Tested**						
		Human rhinovirus	**Influenza B**	***Streptococcus pyogenes (group A Strep)***	**SARS-CoV-2**	**Influenza A**	**Respiratory syncytial virus**	***Streptococcus dysgalactiae (group C/G Strep)***
Human Whole Blood with Na Citrate 10% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Human Sputum/Mucus	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3

1% (v/v)	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Human Genomic DNA 20 ng/µL	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	4/5[a]	5/5	5/5	0/5	0/5	0/5	0/5

Neg: Expected result - Negative for all BIOFIRE SPOTFIRE R/ST Panel analytes.
Pos: Expected result - Positive for representative BIOFIRE SPOTFIRE R/ST Panel analytes highlighted in **red**.
Contrived Positive Samples contained the representative on-panel analytes at 3X LoD.
Contrived Negative Samples contained only the potentially interfering substance.
Testing was performed using the released IVD BIOFIRE SPOTFIRE Respiratory (R) Panel, which is identical in chemical composition to the BIOFIRE SPOTFIRE R/ST Panel Pro used in the remainder of the testing. The data produced was reanalyzed with the Respiratory/Sore Throat Panel v1.1 Panel Software v4.0.1 to provide a consistent analysis for all testing. The data in this table only represents analytes tested in the positive panel and those found in the BIOFIRE SPOTFIRE R/ST Panel Mini.
[a] Initially, 1/3 samples was reported Negative for Human Rhinovirus/Enterovirus; therefore, 2 additional replicates were tested, both of which produced the expected results for all analytes.

Table 6: BIOFIRE SPOTFIRE R/ST Panel Mini Results for Exogenous Substance Interference Testing

Substance Tested Concentration Tested	**Sample**	**Positive Results Reported/Replicates Tested**						
		Human rhinovirus/enterovirus	**Influenza B**	***Streptococcus pyogenes (group A Strep)***	**SARS-CoV-2**	**Influenza A**	**Respiratory syncytial virus**	***Streptococcus dysgalactiae (group C/G Strep)***
Promethazine hydrochloride 1.04 µmol/L (3.34E-04 mg/mL)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Acetaminophen (paracetamol) 1.0E+03 µmol/L (1.5E-01 mg/mL)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Acetylsalicylic acid (Aspirin) 167 µmol/L (3.0E-02 mg/mL)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Ibuprofen 1060 µmol/L (2.2E-01 mg/mL)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	4/5[a]	5/5	5/5	0/5	0/5	0/5	0/5
Albuterol sulfate (ingredient in rescue inhalers) 0.188 µmol/L (5.4E-05 mg/mL)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Triple antibiotic ointment (Neomycin/ polymyxin B/ bacitracin) 2% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3

Mucinex Severe Nasal Congestion Relief Clear & Cool Nasal Spray (Oxymetazoline hydrochloride 0.05%) 1% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Saline nasal spray (sodium chloride 0.65%, disodium phosphate phenylcarbinol, monosodium phosphate and benzalkonium chloride solution) 1% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Vicks VapoRub Cough Suppressant Topical Analgesic (Camphor 4.8%, eucalyptus oil 1.2%, and menthol 2.6%) 1% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Vaseline Petroleum Jelly (100% white petrolatum) 1% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
OrajelTM (0.13% Benzalkonium chloride, benzocaine 20%, menthol 0.5%, zinc chloride 0.15%) 2% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Chloraseptic Sore Throat Spray (Phenol 1.4%) 1% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Vicks Formula 44TM DM (dextromethorphan hydrobromide 0.67mg/mL, guaifenesin 13 mg/mL) (cough syrup) 1% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Phenylephrine hydrochloride (ingredient in nasal decongestants) 1% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Nasal Spray (fluticasone propionate, 50 mcg) 1% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	4/5[a]	5/5	5/5	0/5	0/5	0/5	0/5
Sucrets Sore Throat (dyclonine hydrochloride 2.0 mg/lozenge) 1% w/v	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Benadryl Allergy Liqui-gels (diphenhydramine hydrochloride 25 mg/capsule) 1% (v/v)	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Zicam Cold Remedy (Galphimia Glauca 4x, Luffa Operculata 4x, Sabadilla 4x) 1% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Cold-eeze (zinc gluconate 2.3%) 1% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
HALLS lozenge (menthol 5 mg/lozenge) 1% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Listerine Cool Mint (menthol 0.042%, thymol 0.064%, methyl salicylate 0.06%, eucalyptol 0.092%) 6.5% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Copenhagen Snuff (Tobacco) 1% (w/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	4/5[a]	5/5	5/5	0/5	0/5	0/5	0/5
JUICE HEAD (30% propylene glycol, 70% vegetable glycerin (e-juice)) 1% (v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3

Neg: Expected result - Negative for all BIOFIRE SPOTFIRE R/ST Panel analytes.
Pos: Expected result - Positive for representative BIOFIRE SPOTFIRE R/ST Panel analytes highlighted in red.
Testing was performed using the released IVD BIOFIRE SPOTFIRE Respiratory (R) Panel, which is identical in chemical composition to the BIOFIRE R/ST Panel Pro used in the remainder of the testing. The data produced was reanalyzed with the Respiratory/Sore Throat Panel v1.1 Panel Software v4.0.1 to provide a consistent analysis for all testing. The data in this table only represents analytes tested in the positive panel and those found in the BIOFIRE SPOTFIRE R/ST Panel Mini.
[a] Two out of three replicates reported as positive for Rhinovirus/enterovirus. The unexpected negative result was not reproducible in follow-up testing with two additional replicates.

Table 7: BIOFIRE SPOTFIRE R/ST Panel Mini Results for Disinfectant and Decontaminant Technique-Specific Substances Interference

Substance Tested Concentration Tested	**Sample**	**Positive Results Reported/Replicates Tested**						
		Human rhinovirus/enterovirus	**Influenza B**	***Streptococcus pyogenes (group A Strep)***	**SARS-CoV-2**	**Influenza A**	**Respiratory syncytial virus**	***Streptococcus dysgalactiae (group C/G Strep)***
Bleach (1% v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Bleach (2% v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Bleach- 4-hour incubation (1% v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3

	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Bleach- 18.5-hour (overnight) incubation (1% v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Ethanol (7% v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
Disinfecting wipes (ammonium chloride) 0.25 to 0.5 inch^2/sample	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
DNAZap (1% v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3
RNaseZap™ (1% v/v)	Neg	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Pos	3/3	3/3	3/3	0/3	0/3	0/3	0/3

Neg: Expected result - Negative for all BIOFIRE SPOTFIRE R/ST Panel analytes.
Pos: Expected result - Positive for representative BIOFIRE SPOTFIRE R/ST Panel analytes highlighted in red. Testing was performed using the released IVD BIOFIRE SPOTFIRE Respiratory (R) Panel, which is identical in chemical composition to the BIOFIRE R/ST Panel Pro used in the remainder of the testing. The data produced was reanalyzed with the Respiratory/Sore Throat Panel v1.1 Panel Software v4.0.1 to provide a consistent analysis for all testing. The data in this table only represents analytes tested in the positive panel and those found in the BIOFIRE SPOTFIRE R/ST Panel Mini.

4. Assay Reportable Range:
   Not applicable; this is a qualitative assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   a. Controls
      The assay contains two internal controls (RNA Process Control and PCR2 Control) in each pouch and external positive and negative controls. For more information see **Section IV.C.5 Quality Control**, in the K232954 Decision Summary.

   b. Sample Stability
      Please refer to the Sample Stability Study data previously reviewed and presented in the K232954 FDA Decision Summary.

6. Detection Limit:
   Please refer to the Limit of Detection data previously reviewed and presented in the K232954 FDA Decision Summary.

7. Assay Cut-Off:
   Please refer to the Assay Cut-Off previously reviewed and presented in the K232954 FDA Decision Summary.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable.

2. Matrix Comparison:

This study evaluated equivalence of detection on the BIOFIRE SPOTFIRE R/ST Panel Mini between co-spiked contrived samples prepared in ANS or NPS specimens in Viral Transport Media (VTM). To prepare the sample matrices, ANS and NPS specimens were obtained from asymptomatic donors and placed in VTM. Using pooled ANS and NPS matrix, two contrived mixes were prepared. The sample composition is presented in **Table 8.** A series of three-fold dilutions was prepared with organism concentrations expected to bracket the previously established, isolate-respective LoD. Each sample mix was tested with twenty replicates at each concentration for each sample matrix (NPS and ANS). The dilution series for each analyte included at least one concentration where negative results were observed in two or more replicates (18/20 Positive results). **Table 9** shows concentrations and positivity rate for each organism. Detection by the BIOFIRE SPOTFIRE R/ST Panel Mini was equivalent when samples were prepared in ANS- or NPS-specimen matrices.

Table 8: Sample Mix Composition

Organism	Strain/Isolate	Source ID	Source Concentration	LoD
Sample 1				
Influenza A[c]	Influenza A H1N1 pdm Michigan/45/15	ZeptoMetrix 0810538CF	1.40E+05 $TCID_{50}$/mL	8.2E-01 $TCID_{50}$/mL
Influenza B[c]	B/Florida/02/06 (Victoria Lineage)	ZeptoMetrix 0810037CF	2.50E+05 $TCID_{50}$/mL	3.3E-02 $TCID_{50}$/mL
Human Rhinovirus[a,c]	Human Rhinovirus 1A	ZeptoMetrix 0810012CFN	6.30E+06 $TCID_{50}$/mL	2.1E-01 $TCID_{50}$/mL
SARS-CoV-2[b]	USA-WA1/2020 (heat inactivated)	ATCC VR-1986HK	3.8E+08 copies/mL	2.5E+02 copies/ml
Respiratory syncytial virus[c]	Type B 3/2015 Isolate #1	ZeptoMetrix 0810479CF	4.20E+05 $TCID_{50}$/mL	2.8E-02 $TCID_{50}$/mL
Sample 2				
Influenza A[c]	Influenza A H3N2 A/Hong Kong/4801/14	ZeptoMetrix 0810526CF	7.2E+05 $TCID_{50}$/mL	8.6E-01 $TCID_{50}$/mL
Influenza B[c]	B/Florida/04/06 (Yamagata Lineage)	ZeptoMetrix 0810255CF	1.10E+06 $TCID_{50}$/mL	4.0E-01 $TCID_{50}$/mL
Human Rhinovirus[a,c]	Enterovirus D68 US/MO/14-18947	ATCC VR-1823	1.60E+08 $TCID_{50}$/mL	1.1E+01 $TCID_{50}$/mL
Respiratory syncytial virus[c]	Type A 2006	ZeptoMetrix 0810040ACF	4.20E+05 $TCID_{50}$/mL	6.2E-02 $TCID_{50}$/mL

[a] For the BIOFIRE SPOTFIRE R/ST Panel Mini, both human rhinovirus and enterovirus are reported as "Human rhinovirus."
[b] Heat-inactivated
[c] Live virus

Table 9: Summary of Specimen Comparison Analyte Detection Results

Analyte	Organism Tested Source ID	Test Group	Test Concentration	% Positive (Positive/Tested)	
				ANS	NPS
Human Rhinovirus	Human Rhinovirus 1A	9x LoD	1.9E+00 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		3x LoD	6.3E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1x LoD	2.1E-01 $TCID_{50}$/mL	95% (19/20)	95% (19/20)
		1/3x LoD	7.0E-02 $TCID_{50}$/mL	85% (17/20)	90% (18/20)
		1/9x LoD	2.3E-02 $TCID_{50}$/mL	40% (8/20)	30% (6/20)
		9x LoD	9.9E+01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)

	Human Enterovirus D68a	3x LoD	3.3E+01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1x LoD	1.1E+01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/3x LoD	3.7E+00 $TCID_{50}$/mL	100% (20/20)	95% (19/20)
		1/9x LoD	1.2E+00 $TCID_{50}$/mL	100% (20/20)	80% (16/20)
		1/27x LoD	4.0E-01 $TCID_{50}$/mL	55% (11/20)	65% (13/20)
Influenza A	Influenza A H1N1	9x LoD	7.4E+00 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		3x LoD	2.5E+00 $TCID_{50}$/mL	100% (20/20)	90% (18/20)
		1x LoD	8.2E-01 $TCID_{50}$/mL	95% (19/20)	100% (20/20)
		1/3x LoD	2.7E-01 $TCID_{50}$/mL	85% (17/20)	100% (20/20)
		1/9x LoD	9.1E-02 $TCID_{50}$/mL	85% (17/20)	75% (15/20)
	Influenza A H3N2	9x LoD	7.7E+00 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		3x LoD	2.6E+00 $TCID_{50}$/mL	90% (18/20)	90% (18/20)
		1x LoD	8.6E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/3x LoD	2.9E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/9x LoD	9.6E-02 $TCID_{50}$/mL	95% (19/20)	60% (12/20)
		1/27x LoD	3.2E-02 $TCID_{50}$/mL	40% (8/20)	15% (3/20)
Influenza B	Influenza B (Victoria Lineage)	9x LoD	3.0E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		3x LoD	9.9E-02 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1x LoD	3.3E-02 $TCID_{50}$/mL	95% (19/20)	100% (20/20)
		1/3x LoD	1.1E-02 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/9x LoD	3.7E-03 $TCID_{50}$/mL	90% (18/20)	85% (17/20)
	Influenza B (Yamagata Lineage)	9x LoD	3.6E+00 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		3x LoD	1.2E+00 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1x LoD	4.0E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/3x LoD	1.3E-01$TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/9x LoD	4.4E-02 $TCID_{50}$/mL	95% (19/20)	75% (15/20)
		1/27x LoD	1.5E-02 $TCID_{50}$/mL	45% (9/20)	70% (14/20)
Respiratory syncytial virus	Respiratory syncytial virus Type A	9x LoD	5.6E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		3x LoD	1.9E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1x LoD	6.2E-02 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/3x LoD	2.1E-02 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/9x LoD	6.9E-03 $TCID_{50}$/mL	95% (19/20)	90% (18/20)
		1/27x LoD	2.3E-03 $TCID_{50}$/mL	50% (10/20)	55% (11/20)
	Respiratory syncytial virus Type B	9x LoD	2.5E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		3x LoD	8.4E-02 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1x LoD	2.8E-02 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
		1/3x LoD	9.3E-03 $TCID_{50}$/mL	100% (20/20)	95% (19/20)
		1/9x LoD	3.1E-03 $TCID_{50}$/mL	75% (15/20)	85% (17/20)
SARS-CoV-2	SARS-CoV-2	9x LoD	2.3E+03 copies/mL	100% (20/20)	100% (20/20)
		3x LoD	7.5E+02 copies/mL	100% (20/20)	95% (19/20)
		1x LoD	2.5E+02 copies/mL	100% (20/20)	100% (20/20)
		1/3x LoD	8.3E+01 copies/mL	90% (18/20)	95% (19/20)
		1/9x LoD	2.8E+01 copies/mL	60% (12/20)	35% (7/20)

Shaded cells indicate the lowest concentration where at least 95% of samples returned Positive.

C Clinical Studies:

1. Clinical Sensitivity:

Prospective Clinical Study

The clinical performance of the BIOFIRE SPOTFIRE R/ST Panel Mini when testing ANS specimens was established during a prospective multi-center study. Five geographically distinct study sites located in the U.S. and representative of the intended use setting participated in this study from March 2024 to February 2025.

A total of 820 ANS specimens were initially enrolled in the prospective clinical study; 23 of these were excluded from all analyses. Eleven (11) specimens were excluded because no valid BIOFIRE SPOTFIRE R/ST Panel Mini test for the ANS specimen was obtained, eight specimens were excluded because no valid comparator test was obtained, and four additional specimens were excluded due to those specimens having been determined to not meet the inclusion criteria after initial enrollment. The final data set consisted of 797 specimens. Additionally, six of the 797 specimens were excluded from the evaluation of influenza A due to initial "Uncertain" results that were not appropriately retested by operators at study sites (N=4) or initial inconclusive comparator results that were unable to be retested due to insufficient volume (N=2). For each analyte, the performance of the BIOFIRE SPOTFIRE R/ST Panel Mini when testing ANS specimens was evaluated by comparing the test results with those from an FDA-cleared test.

The overall success rate for initial specimen testes was 97.0% (782/806). Nine tests (9/806; 1.1%) did not complete on the initial run, resulting in a total instrument success rate of 98.9% (797/806) for initial specimen tests. All nine specimens were able to be retested and valid results were produced on the first (eight specimens) or second (one specimen) retest.

The demographics and performance of the BIOFIRE SPOTFIRE R/ST Panel Mini for the ANS sample type are summarized in **Table 10** and **Table 11** respectively. Positive percent agreement (PPA) for each analyte was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the BIOFIRE SPOTFIRE R/ST Panel Mini and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the BIOFIRE SPOTFIRE R/ST Panel Mini was negative while the comparator result was positive. Negative percent agreement (NPA) was calculated as 100% × (TN / TN + FP)). True negative (TN) indicates that both the BIOFIRE SPOTFIRE R/ST Panel Mini and the comparator method had negative results, and false positive (FP) indicates that the BIOFIRE SPOTFIRE R/ST Panel Mini was positive while the comparator method was negative.

Table 10: Demographic Summary for the BIOFIRE SPOTFIRE R/ST Panel Mini Prospective Clinical Evaluation

Category		**N=797**
Age	≤5 years	240 (30.1%)
	6-18 years	213 (26.7%)
	19-40 years	178 (22.3%)
	41-60 years	116 (14.6%)
	61+ years	50 (6.3%)
Total		797

Table 11: BIOFIRE SPOTFIRE R/ST Panel Mini Prospective Clinical Performance Summary for ANS Specimens

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
SARS-CoV-2[a]	50/52	96.2	87.0-98.9	742/745	99.6	98.8-99.9
Human rhinovirus[b]	222/232	95.7	92.2-97.6	537/565	95.0	92.9-96.5
Influenza A[c]	50/53	94.3	84.6-98.1	738/738	100	99.5-100.0
Influenza B	13/13	100	77.2-100.0	784/784	100	99.5-100.0
RSV[d]	38/40	95.0	83.5-98.6	756/757	99.9	99.3-100.0

[a] SARS-CoV-2 was detected 1/3 FP specimens when tested with additional molecular methods.
[b] Human rhinovirus was detected in 1/28 FP specimens using an additional molecular method.
[c] Influenza A virus was detected in the three FN specimens using an additional molecular method.
[d] Respiratory syncytial virus was detected in the single FP specimen using an additional molecular method.

The BIOFIRE SPOTFIRE R/ST Panel Mini reported a total of 21 ANS specimens with multiple organism detections (2.6% of all ANS specimens, 21/805; 5.5% of positive ANS specimens, 21/385). The resulting co-detection combinations as reported by the BIOFIRE SPOTFIRE R/ST Panel Mini are presented in **Table 12**. This table also indicates the number of specimens with false positive (FP) results for each co-detection combination, as well as the specific analyte(s) that were discrepant.

Table 12: Multiple Detection Combinations in ANS Specimens as Reported by the BIOFIRE SPOTFIRE R/ST Panel Mini

Distinct Co-Detection Combinations		Total Specimens with Co-Detections	Number of Specimens with False Positive Co- Detections	False Positive Analyte(s)
Analyte 1	Analyte 2			
SARS-CoV-2	Human rhinovirus	8	1	SARS-CoV-2 (1), Human rhinovirus (1)
SARS-CoV-2	Respiratory syncytial virus	1	0	-
Human rhinovirus	Influenza A	9	2	Human rhinovirus (2)
Human rhinovirus	Respiratory syncytial virus	3	1	Human rhinovirus (1)
Total Co-Detections		**21**	**4**	**5/42[a]**

[a] Of the five discrepant analytes (out of 42 total analytes), one was confirmed as being present in the specimen during discrepancy investigation utilizing an FDA-cleared molecular assay.

Retrospective Clinical Study

To supplement the results of the prospective clinical study for influenza B, which was of low prevalence during the prospective clinical study, an evaluation of preselected archived retrospective anterior nasal swab (ANS) specimens was performed. Archived clinical specimens consisted of residual leftover ANS in VTM that were obtained from one clinical laboratory and one specimen biorepository. A total of 242 (35 positive and 207 negative) frozen archived ANS specimens were obtained from these two external sites and were retrospectively tested at an internal site. All 242 ANS specimens had valid results that were included in performance analysis. Demographics and the performance results for archived specimens are shown in **Table 13** and **Table 14** respectively. Comparator testing was performed using an acceptable FDA cleared molecular assay.

Table 13: Demographic Summary for All Valid Archived ANS Specimens

Total Specimens		N=242
Age Range	<5 year	108 (44.6%)
	6-18 years	121 (50.0%)
	19-40 years	6 (2.5%)
	41-60 years	5 (2.1%)
	61+ years	2 (0.8%)

Table 14: BIOFIRE SPOTFIRE R/ST Panel Mini Archived Clinical Performance Summary for ANS

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
Influenza B	35/35	100	90.1-100.0	206/206[a]	100	98.2-100.0

[a] One of the 207 negative specimens was unexpectedly identified as positive by the confirmatory molecular method and was excluded.

2. Clinical Specificity:
   Refer to Clinical Sensitivity above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

In the prospective clinical evaluation of anterior nasal swab (ANS) specimens as a sample type for use with the BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini, 797 ANS specimens were evaluated for SARS-CoV-2, influenza A, influenza B, RSV, and human rhinovirus. Expected value summaries are stratified by specimen enrollment site and subject age group in **Table 15** and **Table 16**, respectively.

Table 15: Expected Value Summary by Site for ANS Specimens Collected During the BIOFIRE SPOTFIRE R/ST Panel Mini ANS Prospective Clinical Evaluation (March 2024 to February 2025)

BIOFIRE SPOTFIRE R/ST Panel Mini R Menu Result	Overall		Site 1		Site 2		Site 3		Site 4		Site 5	
	#/SA[a]	EV[b]	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV	#/SA	EV
SARS-CoV-2	**53/797**	**6.6%**	3/67	4.5%	18/197	9.1%	15/151	9.9%	13/253	5.1%	4/129	3.1%
Human rhinovirus	**250/797**	**31.4%**	26/67	38.8%	55/197	27.9%	38/151	25.2%	93/253	36.8%	38/129	29.5%
Influenza A	**51/797**	**6.4%**	6/67	9.0%	17/197	8.6%	8/151	5.3%	11/253	4.3%	9/129	7.0%

Influenza B	**13/797**	**1.6%**	3/67	4.5%	9/197	4.6%	0/151	0%	1/253	0.4%	0/129	0%
RSV	**39/797**	**4.9%**	1/67	1.5%	6/197	3.0%	0/151	0%	7/253	2.8%	25/129	19.4%

[a] #/SA = number of BIOFIRE SPOTFIRE R/ST Panel Mini positives divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte
[b] EV = Expected Value

Table 16: Expected Value Summary by Age Group for ANS Specimens Collected During the BIOFIRE SPOTFIRE R/ST Panel Mini ANS Prospective Clinical Evaluation (March 2024 to February 2025)

BIOFIRE SPOTFIRE R/ST Panel Mini R Menu Result	**Overall**		**≤5 years**		**6-18 years**		**19-40 years**		**41-60 years**		**61+ years**	
	#/SA[a]	**EV[b]**	**#/SA**	**EV**	**#/SA**	**EV**	**#/SA**	**EV**	**#/SA**	**EV**	**#/SA**	**EV**
SARS-CoV-2	**53/797**	**6.6%**	8/240	3.3%	13/213	6.1%	17/178	9.6%	13/116	11.2%	2/50	4.0%
Human rhinovirus	**250/797**	**31.4%**	106/240	44.2%	54/213	25.4%	53/178	29.8%	28/116	24.1%	9/50	18.0%
Influenza A	**51/797**	**6.4%**	13/240	5.4%	13/213	6.1%	12/178	6.7%	9/116	7.8%	4/50	8.0%
Influenza B	**13/797**	**1.6%**	0/240	0%	4/213	1.9%	7/178	3.9%	1/116	0.9%	1/50	2.0%
Respiratory syncytial virus	***39/797***	***4.9%***	30/240	12.5%	3/213	1.4%	1/178	0.6%	4/116	3.4%	1/50	2.0%

[a] #/SA = number of BIOFIRE SPOTFIRE R/ST Panel Mini positives divided by the number of specimens analyzed (i.e., with valid comparator results) for that analyte
[b] EV = Expected Value

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.