Appendix C: Infringement Claim Chart for U.S. Patent No. 11,827,921

Plaintiffs provide in this chart exemplary allegations that Bio-Rad's ddPCR-related assays and kits infringe at least Claims 1-17 and 19 of U.S. Patent No. 11,827,921 ("the '921 Patent"). On information and belief, such infringing assays and kits include (but are not limited to):

- Bio-Rad's SARS-CoV-2 ddPCR Kit ("SARS-CoV-2 Kit");
- Bio-Rad's PREvalence ddPCR SARS-CoV-2 Wastewater Quantification Kit ("SARS-CoV-2 Wastewater Quantification Kit");
- Bio-Rad's ddPCR Microsatellite Instability (MSI) Kit (including ddPCR MSI Assay 1 (BAT25/BAT26), ddPCR MSI Assay 2 (NR21/NR24)) ("MSI Kit");
- Bio-Rad's Expert Design Assay ID dHsaEXD42003661 (EGFR C797S Multiplex Tube 1) ("The '3661 Assay");
- Bio-Rad's Expert Design Assay ID dHsaEXD41810842, the EGFR C797S T790M L858R Multiplex ("The '0842 Assay"); and
- Bio-Rad's Expert Design Assay ID dEXD84844642, the Influenza A/B Triplex Assay ("The '4642 Assay").[1]

As non-limiting examples, Plaintiffs identify how the foregoing kits and assays are representative of Bio-Rad's infringing ddPCR assays and kits. Plaintiff's investigation into additional infringing assays and kits is ongoing and Plaintiffs investigation into additional assays and kits that may infringe is ongoing. Plaintiffs show below how the examples identified above meet every limitation in the below claimed system.

Bio-Rad's ddPCR-related assays and kits are designed to be used in connection with various devices, software, reagents, consumables, and other accessories including, but not limited to:

- All devices, accessories, consumables, and/or components identified in Appendices A-01, A-02, B-01, and B-02; and
- All Devices (QX200 ddPCR system, QX200 droplet generator, and QX200 droplet reader, QX Manager software (standard/premium)), Accessories (PX1 PCR Plate Sealer, C1000 Touch Thermal Cycler, PTC Tempo Deepwell Thermal Cycler), Kits & Reagents, and Consumables identified for use with the QX200 ddPCR system at, for example, https://www.bio-rad.com/en-us/life-science/digital-pcr/qx200-droplet-digital-pcr-system.

[1] Hereafter, the "Accused Products" refer to Bio-Rad's ddPCR-related Kits and Assays as exemplified by the SARS-CoV-2 Kit, SARS-CoV-2 Wastewater Quantification Kit, dHsaEXD42003661 Assay, the MSI Kit, dHsaEXD41810842 Assay and dEXD84844642 Assay collectively.

BIO-RAD EX.1012.001

Bio-Rad directly infringes the Asserted Claims by using the Accused Products alone and/or with any of the compatible devices, software, accessories, and/or components to perform every claim limitation of the claimed system below.

Bio-Rad also indirectly infringes the Asserted Claims by providing the Accused Products. Bio-Rad also induces customers and/or end users to use the Accused Product to infringe by inducing them to use these infringing products in violation of 35 U.S.C. § 271(b).

Bio-Rad also contributes to the infringement of customers and/or end-users by providing the Accused Products to the same who then use the Accused Products in connection with other devices, components, and/or consumables (such as those identified above) in violation of 35 U.S.C. § 271(c). By way of non-limiting example, such customers and/or end users use the Accused Products to practice each and every limitation of the Asserted Claims.

On information and belief, Bio-Rad also supplies or causes to be supplied in or from the United States all or a substantial portion of the Accused Products such that Bio-Rad induces infringe of customers or end users outside the United States in violation of 35 U.S.C. § 271(f)(1). For like reasons as described above, Bio-Rad contributes to the infringement of customers or end users outside the United States in violation of 35 U.S.C. § 271(f)(2).

Plaintiffs allege infringement both literally and under the doctrine of equivalents.

Claims	Exemplary Disclosure of Accused Products
[1-PRE] **1. An assay that is capable of unambiguously detecting the presence or absence of each of at least M analytes,**	To the extent that this preamble is limiting, the Accused Products include assays that are capable of unambiguously detecting the presence or absence of each of at least M analytes either literally or under the doctrine of equivalents. This capability is realized when the Accused Products are used in connection with the various devices and systems listed above, including without limitation the QX200, QX600, QX ONE, Plate Sealers, thermal cyclers, and other reagents and components. The Accused Products (as represented in the following exemplary assays and kits) practice Claim 1's preamble.

BIO-RAD EX.1012.002

SARS-CoV-2 Kit

The SARS-CoV-2 Kit can unambiguously detect the presence or absence of three analytes: 2019-nCoV (N1, N2) genes, and the human RNase P gene (RP):

The Bio-Rad SARS-CoV-2 ddPCR Kit is a reverse transcription (RT) droplet digital polymerase chain reaction (ddPCR) test designed to detect RNA from the SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate swabs, nasopharyngeal washes/aspirates and nasal aspirates, as well as bronchoalveolar lavage (BAL) specimens from patients suspected of COVID-19 by their healthcare provider.

The oligonucleotide primers and probes for detection of SARS-CoV-2 are the same as those reported by CDC and were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel. The Bio-Rad SARS-CoV-2 ddPCR Kit includes these three sets of primers/probes into a single assay multiplex to enable a one-well reaction.

The sample and mastermix RT-ddPCR mixtures are fractionated into up to 20,000 nanoliter-sized droplets in the form of a water-in-oil emulsion in the Automated Droplet Generator. The emulsions are then thermocycled to achieve reverse transcription to generate cDNA followed by target amplification plus probe hydrolysis in each droplet. Subsequent to PCR, the fluorescence intensity of each droplet is measured in two channels (FAM and HEX) in the Droplet Reader. The fluorescence data is then analyzed by the QuantaSoft v1.7 Software and QuantaSoft Analysis Pro v1.0 Software to determine the presence of SARS-CoV-2 N1 and N2 in the specimen.

After thermocycling is complete, the 96-well RT-ddPCR ready plate is loaded into the QX200/QXDx Droplet Reader. The Droplet Reader singulates the droplets and flows them past a two-color fluorescence detector. The detector reads the droplets to determine which contain target (positive) and which do not (negative) for each of the targets identified with the SARS-CoV-2 ddPCR Kit: N1, N2 and RP. The ddPCR system uses the QX Manager 1.1, or QuantaSoft 1.7 and QuantaSoft Analysis Pro 1.0 for analysis software.

BIO_CCODE00006914 at 6930.

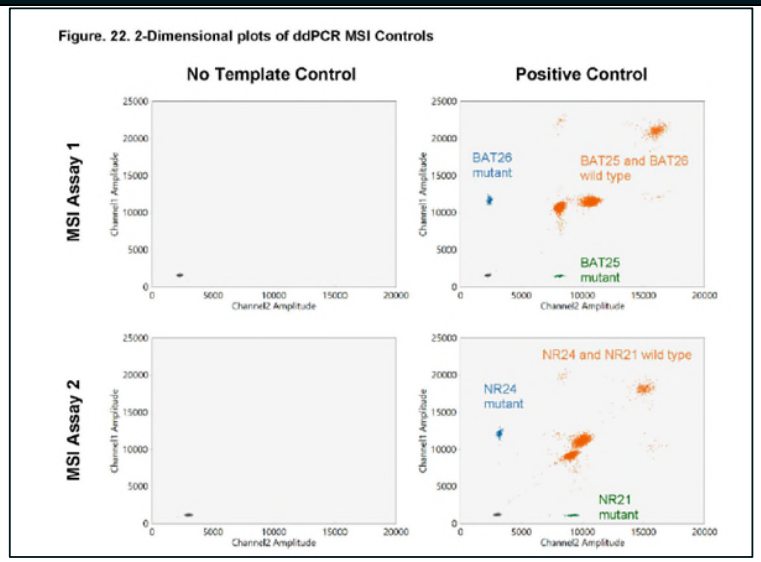
SARS-CoV-2 Wastewater Quantification Kit

The SARS-CoV-2 Wastewater Quantification Kit can unambiguously detect the presence or absence of three analytes: SARS-CoV-2 (N2, E) genes, and the murine coronavirus control, murine hepatitis virus (MHV) gene:

BIO-RAD EX.1012.003

Claims	Exemplary Disclosure of Accused Products
	Testing wastewater for SARS-CoV-2 enables traceability and pre-emptive surveillance. This allows regions to determine viral loads within the sewage system and track the amount over time to predict viral outbreaks. This information can then be brought to the attention of health authorities for prompt implementation of safety guidelines. The PREvalence ddPCR SARS-CoV-2 Wastewater Quantification Kit, based on the U.S. Centers for Disease Control and Prevention and World Health Organization RT-PCR primers for SARS-CoV-2 detection, allows for the detection of N2 and E genes in SARS-CoV-2. It also includes an assay for a murine coronavirus control, murine hepatitis virus (MHV), which is an enveloped, single-stranded RNA virus. MHV serves as a matrix recovery control, enabling estimation of the virus lost during sample processing. The test allows for all three targets to be detected in a single well using the kit. Droplet Digital PCR is a quantitative, endpoint PCR that partitions a sample into approximately 20,000 individual droplets and allows for higher sensitivity and greater resistance to inhibition compared to other types of quantitative PCR (qPCR) methods. Each droplet is an individual PCR reaction that is counted as positive or negative after PCR amplification. The result is a quantitative description of the number of template copies in the total PCR reaction. This technology also yields high reproducibility and low variability. BIO_CCODE00006969 at 6971. **<u>MSI Kit</u>** The MSI Kit Assay 1 can unambiguously detect the presence or absence of the three analytes: BAT25, BAT26, BAT25 and BAT26 wild type. The MSI Kit Assay 2 can unambiguously detect the presence or absence of the three analytes: NR21, NR24, NR21 and NR24 wild type.

Claims	Exemplary Disclosure of Accused Products
	Section 1 **Introduction** Microsatellites are short tandem repeats of DNA, with repeat units that are typically 1 to 6 bp in length and repeated 5 to 50 times. They are distributed throughout the genome and are more susceptible to mutations. Microsatellite instability (MSI) arises when microsatellites undergo a length change as a result of an impaired mismatch repair (MMR) system. Approximately 15% of colorectal cancer patients exhibit an impaired MMR system and MSI, stemming from either somatic or germline mutations in MMR genes (Boland and Goel 2010). Studies have shown patients with MSI typically have a better prognosis and are more likely to respond to immunotherapy, underscoring the need for a test that can assess MSI (Dudley 2016, Vilar and Gruber 2010). The ddPCR Microsatellite Instability Kit detects deletions and insertions in five mononucleotide microsatellite markers (BAT25, BAT26, NR21, NR24, and Mono27) using Droplet Digital PCR technology, in plasma or tumor tissue, including formalin-fixed paraffin-embedded (FFPE) tissue from colorectal cancer patients and other potentially MSI-relevant cancers. These five markers have been classified as sensitive markers to size alterations and shifts in microsatellites in tumors with defective MMR systems (Bacher, et al. 2004). The ddPCR MSI Kit consists of three assays: assay 1 (BAT25 and BAT26), assay 2 (NR21 and NR24), and assay 3 (Mono27). This test does not assess the mutational status of MMR genes or whether any such mutations are somatic or germline. The ddPCR Microsatellite Instability Kit includes enough reagents for 200 reactions to test up to 56 samples including controls. The kit is intended for use on the Bio-Rad™ QX200™ Droplet Digital PCR System or the QX200 AutoDG™ Droplet Digital PCR System. BIO_CCODE00006995 at 6997.

Claims	Exemplary Disclosure of Accused Products
	 BIO_CCODE00006995 at 7019. **<u>The ’3661 Assay</u>** The dHsaEXD42003661 Assay can unambiguously detect the presence or absence of three analytes: EGFR C797S G>C, C797S T>A, and C797S WT variants. **<u>Expert Design Assay: Unique Assay ID dHsaEXD42003661</u>** **<u>Description</u>** The *EGFR* T790M/C797S/L858R multiplex assay, provided in 2 separate tubes, is a 20X assay designed to detect four EGFR mutations, T790M, L858R, C797S G>C, and C797S T>A, in two wells, using the QX 200™ Droplet Digital™ PCR System. Tube 1 (dHsaEXD42003661) detects EGFR C797S G>C, C797S T>A and C797S WT.

Claims	Exemplary Disclosure of Accused Products
	BIO_CCODE00008545 at 8545. **<u>The ’0842 Assay</u>** The dHsaEXD41810842 Assay can unambiguously detect the presence or absence of the five analytes: T790M, L858R, C797S G>C, C797S T>A and C797 Wild Type: **<u>Expert Design Assay: Unique Assay ID dHsaEXD41810842</u>** **<u>Description</u>** The EGFR Multiplex Assay is a 20X assay designed to discriminate EGFR; T790M, L858R, C797S T>A, and C797S G>C variants using Bio-Rad's QX ONE Droplet Digital PCR system. This assay should be validated prior to use by the end-user. **<u>Targets Detected by the Assay</u>** EGFR Variant Name | Cosmic ID | Fluorophore | Amplicon Length T790M | COSM6240 | FAM | 98 L858R | COSM6224 | HEX | 73 C797S G>C | COSM5945664 | Cy5 | 98 C797S T>A | COSM6493937 | Cy5/Cy5.5 | 98 C797 Wild type | N/A | Cy5.5 | 98 https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf at 1.

Claims	Exemplary Disclosure of Accused Products
	Other Reagents Required ddPCR Multiplex Supermix, 2 x 0.5ml – cat# 12005909 **Host** Homo sapiens **ddPCR Reaction Setup** <table><tr><th>Component</th><th>Volume per Reaction, µl</th><th>Final Concentration</th></tr><tr><td>4x ddPCR Multiplex Supermix</td><td>5</td><td>1x</td></tr><tr><td>20x EGFR Multiplex Assay</td><td>1</td><td>1x</td></tr><tr><td>Restriction enzyme, diluted*</td><td>1</td><td>5-10U per reaction</td></tr><tr><td>DNA sample or water</td><td>Up to 13ul</td><td>Variable</td></tr><tr><td>**Total Volume**</td><td>**20****</td><td>**–**</td></tr></table>* When not digesting directly in the ddPCR reaction setup this component can be replaced by water ** For the Automated Droplet Generator, prepare 22 ul per well. https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf at 2. **The ’4642 Assay** The dEXD84844642 Assay can unambiguously detect the presence or absence of three analytes: Influenza A, Influenza B, and Human RPP30 genes:

Claims	Exemplary Disclosure of Accused Products
	Expert Design Assay: Unique Assay ID dEXD84844642 **Description** The Influenza A/B ddPCR Triplex Probe Assay is a 20X assay designed to detect Influenza A, Influenza B, and Human RPP30 genes using Droplet Digital PCR. This assay should be validated prior to use by the end-user. **Targets Detected by the Assay** Variant Name | ID | Fluorophore | Amplicon Length Influenza A | N/A | FAM | 106 Influenza B | N/A | FAM/HEX | 103 Rpp30 | ENST00000487998.5 | HEX | 103 BIO_CCODE00008542 at 8542.
[1a] **wherein each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,**	Bio-Rad's infringing ddPCR assays and kits each encode at least M analytes as a value of one component of fluorescent signal in the form of a specific wavelength as defined by the fluorophore either literally or under the doctrine of equivalents. For each infringing product, each of the M analytes is associated with one or more fluorophores. Each fluorophore corresponds to a specific wavelength of light when excited. When a fluorophore-labeled probe is in contact with the associated analyte, the fluorophore becomes excited and emits light. Through this association, each analyte becomes encoded as a value of one component of a fluorescent signal. A coding scheme follows based on how the Accused Products are encoded. The following are representative examples of how this occurs:

BIO-RAD EX.1012.009

SARS-CoV-2 Kit

In the SARS-CoV-2 Kit, each analyte is encoded as a value of one component of a fluorescence signal (corresponding to a specific wavelength as defined by the fluorophore given below) as represented by the identified fluorophores FAM and HEX:

4. In the **Plate Editor** tab, select all the wells to be analyzed. With **Experiment Type** as **Direct Quantification (DQ)**; select **Probe Mix Triplex** under **Assay Information** and then click **Apply**. Enter Target information (Table 10) and click **Apply**.

Table 10. QuantaSoft Analysis Pro Target Information

Target Name	Signal Ch1	Signal Ch2
N1	FAM	None
N2	FAM	HEX
RP	None	HEX

BIO_CCODE00006914 at 6937.

In the SARS-CoV-2 Kit, N1 is encoded as FAM positive, N2 as FAM and HEX positive, and RP as HEX positive. As explained above, the detector reads the droplet to determine which contain target (positive – 1), and which do not (negative – 0) for each of the targets identified with the SARS-CoV-2 Kit. The corresponding coding scheme for the SARS-CoV-2 Kit is as follows:

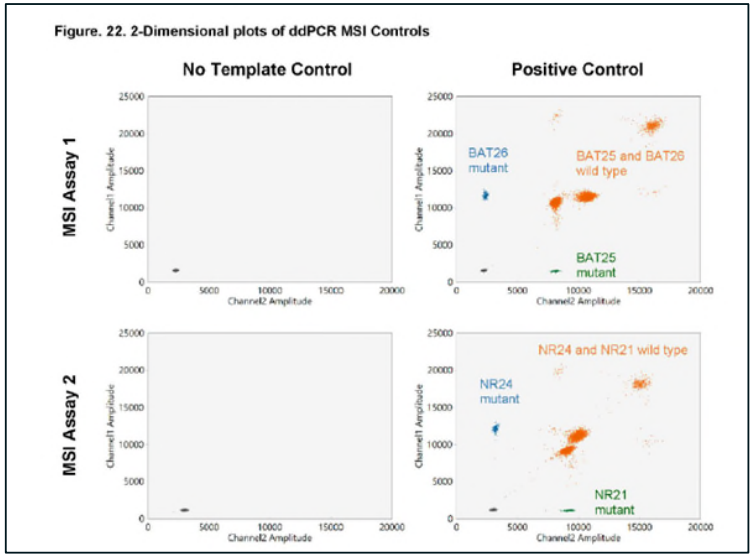
Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

SARS-CoV-2 Wastewater Quantification Kit

In the SARS-CoV-2 Wastewater Quantification Kit, each target (*i.e.*, the analyte) is associated with a fluorophore (corresponding to a specific wavelength). Each analyte is detected based on the paired fluorophore emitting a fluorescent signal of its corresponding wavelength. Exemplary fluorophores include FAM and HEX:

BIO-RAD EX.1012.010

Claims	Exemplary Disclosure of Accused Products
	Using QX Manager Software Standard Edition, set up a plate run, being sure to use the following plate and well parameters: ▪ Experiment Type: **Direct Quantification (DQ)** ▪ Sample Type: **Unknown** for samples, **NTC** for NTC, and **Pos Ctrl** for the RT-ddPCR control ▪ Supermix: **One-Step RT-ddPCR Advanced Kit for Probes** ▪ Assay Type: **Probe Mix Triplex** ▪ Targets: – N2 (Signal Ch1: **FAM**; Signal Ch2: **None**) – E (Signal Ch1: **FAM**; Signal Ch2: **HEX**) – MHV (Signal Ch1: **None**; Signal Ch2: **HEX**) BIO_CCODE00006971 at 6979. In the SARS-CoV-2 Wastewater Quantification Kit, N2 is encoded as FAM positive, E as FAM and HEX positive, and NHV as HEX positive. As explained above, the detector reads the droplet to determine which contain target (positive – 1), and which do not (negative – 0) for each of the targets identified with the SARS-CoV-2 Wastewater Quantification Kit. The corresponding coding scheme for the SARS-CoV-2 Wastewater Quantification Kit is shown below. **Analyte FAM HEX** **N2** 1 0 **E** 1 1 **MHV** 0 1 **MSI Kit** In the MSI Kit Assay 1: • BAT26 mutant is encoded as a positive FAM fluorescent signal; • BAT25 mutant is encoded as a positive HEX fluorescent signal;

Claims	Exemplary Disclosure of Accused Products
	• BAT25 and BAT26 wild type are encoded as a positive of FAM fluorescent signal and a positive HEX fluorescent signal; In the MSI Kit Assay 2: • NR24 mutant is encoded as a positive FAM fluorescent signal; • NR21 mutant is encoded as a positive HEX fluorescent signal; • NR24 and NR21 wild type are encoded as a double-positive of both FAM and HEX fluorescent signals.  BIO_CCODE00006995 at 7019. The corresponding coding scheme for the MSI Kit is shown below: **Assay 1 Analyte** / **FAM** / **HEX** **BAT26 mutant** / 1 / 0

Claims	Exemplary Disclosure of Accused Products

BAT25 mutant	0	1
BAT25 and BAT26 wild type	1	1

Assay 2 Analyte	FAM	HEX
NR24 mutant	1	0
NR21 mutant	0	1
NR24 and NR21 wild type	1	1

The ’3661 Assay

In the dHsaEXD42003661 Assay, each target is paired with a fluorophore as defined in the instructions below (FAM, FAM/HEX, and HEX). Each analyte is detected as a value of one component of a fluorescence signal (e.g., a specific wavelength as defined by the fluorophore given below).

Targets Detected by the Assay

Variant Name	COSMIC ID	Fluorophore	Amplicon Length
EGFR C797S G>C	COSM5945664	FAM	98
EGFR C797S T>A	COSM6493937	FAM/HEX	98
EGFR C797S WT	N/A	HEX	98

BIO_CCODE00008545 at 8545.

Each analyte is encoded as a fluorescence value (i.e., a specific wavelength as defined by the fluorophore given above). EGFR C797S G>C is encoded as FAM positive. EGFR C797S WT is encoded as HEX positive. EGFR C797S T>A is encoded as FAM and HEX positive. As explained above, the detector reads the droplet to determine which contain target (positive – 1), and which do not

Claims	Exemplary Disclosure of Accused Products

(negative – 0) for each of the targets identified with the dHsaEXD42003661 Assay. The corresponding coding scheme is shown below:

Analyte	FAM	HEX
EGFR C797S G>C	1	0
EGFR C797S T>A	1	1
EGFR C797S WT	0	1

The '0842 Assay

In the dHsaEXD41810842 Assay, T790M is encoded as FAM positive; L858R is encoded as HEX positive; C797S G>C is encoded as Cy5 positive; C797S T>A is encoded as Cy5 and Cy5.5 positive; and C797 Wild type is encoded as Cy5.5 positive:

Targets Detected by the Assay

EGFR Variant Name	Cosmic ID	Fluorophore	Amplicon Length
T790M	COSM6240	FAM	98
L858R	COSM6224	HEX	73
C797S G>C	COSM5945664	Cy5	98
C797S T>A	COSM6493937	Cy5/Cy5.5	98
C797 Wild type	N/A	Cy5.5	98

https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf, at 1.

Each analyte is encoded as a fluorescence value (i.e., a specific wavelength as defined by the fluorophore given above). T790M is encoded as FAM positive. L858R is encoded as HEX positive. C797S G>C is encoded as Cy5 positive. C797S T>A is encoded as Cy5 and Cy5.5 positive. C797 Wild type is encoded as Cy5.5 positive. Droplets that contain target is indicated as positive – 1, and droplets that do not contain target is indicated as negative – 0. The corresponding coding scheme is shown below:

Claims	Exemplary Disclosure of Accused Products

Analyte	FAM	HEX	Cy5	Cy5.5
T790M	1	0	0	0
L858R	0	1	0	0
C797S G>C	0	0	1	0
C797S T>A	0	0	1	1
C797S Wild Type	0	0	0	1

The '4642 Assay

In the dEXD84844642 Assay, Influenza A is encoded as FAM positive; Influenza B is encoded as FAM and HEX positive, and RPP30 is encoded as HEX positive:

Targets Detected by the Assay

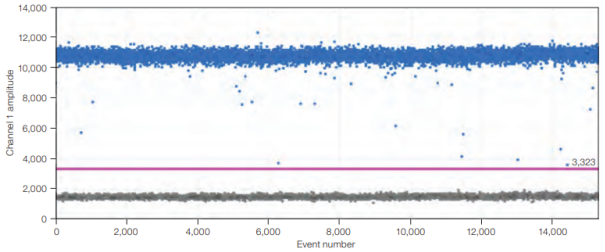
Variant Name	ID	Fluorophore	Amplicon Length
Influenza A	N/A	FAM	106
Influenza B	N/A	FAM/HEX	103
Rpp30	ENST00000487998.5	HEX	103

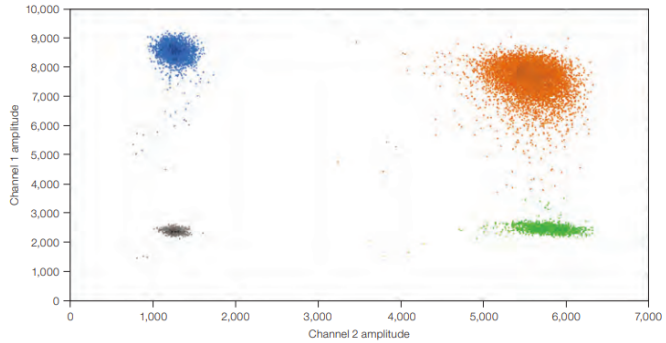
BIO_CCODE00008542 at 8542.

Each analyte is encoded as a fluorescence value (i.e., a specific wavelength as defined by the fluorophore given above). Influenza A is encoded as FAM positive. Influenza B is encoded as FAM and HEX positive. RPP30 is encoded as HEX positive. Droplets that contain target is indicated as positive – 1, and droplets that do not contain target is indicated as negative – 0. The corresponding coding scheme is shown below:

BIO-RAD EX.1012.015

Claims	Exemplary Disclosure of Accused Products
	<table><tr><th>Analyte</th><th>FAM</th><th>HEX</th></tr><tr><td>**Influenza A**</td><td>1</td><td>0</td></tr><tr><td>**Influenza B**</td><td>1</td><td>1</td></tr><tr><td>**Rpp30**</td><td>0</td><td>1</td></tr></table>
[1b] **wherein said fluorescent signal comprises F wavelength components, and**	For Bio-Rad's infringing products, the encoding scheme identifies how many wavelength components correspond to each analyte's encoded fluorescent signal. For example, as shown in the exemplary assays and kits described above: • In the SARS-CoV-2 Kit, the fluorescence signal comprises two components as discussed in [1a] (i.e., FAM channel to detect FAM fluorophores and HEX channel to detect HEX fluorophores). Thus, F = 2. *See* BIO_CCODE00006914 at 6937. • In the SARS-CoV-2 Wastewater Quantification Kit, the fluorescence signal comprises two components as discussed in [1a] (i.e., FAM channel to detect FAM fluorophores and HEX channel to detect HEX fluorophores). Thus, F = 2. *See* BIO_CCODE00006971 at 6979. • In the MSI Kit, the fluorescent signal comprises two wavelength components as discussed in [1a] (i.e., FAM channel to detect FAM fluorophores and HEX channel to detect HEX fluorophores). Thus, F = 2. *See* BIO_CCODE00006995 at 7019. • In the '3661 Assay, the fluorescence signal comprises two components as discussed in [1a] (i.e., FAM channel to detect FAM fluorophores and HEX channel to detect HEX fluorophores). Thus, F = 2. *See* BIO_CCODE00008545 at 8545. • In the '0842 Assay, the fluorescent signal comprises four wavelength components (i.e., FAM channel to detect FAM fluorophores and HEX channel to detect HEX fluorophores, Cy5 channel to detect Cy5 fluorophores, and Cy5.5 channel to detect Cy5.5 fluorophores). Thus, F = 4. *See* https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf at 1-4.

Claims	Exemplary Disclosure of Accused Products
	• In the ’4642 Assay, the fluorescent signal comprises two wavelength components (i.e., FAM channel to detect FAM fluorophores and HEX channel to detect HEX fluorophores). Thus, F = 2. *See* BIO_CCODE00008542 at 8542.
[1c] **wherein said assay is capable of unambiguously detecting the presence or absence of each of said at least M analytes, in any combination of presence or absence, in a single sample volume, based on detection of said F wavelength components, when M is greater than F,**	Bio-Rad’s infringing ddPCR assays and kits are able to unambiguously detect the presence or absence of each of the analytes, in any combination of presence or absence. This detection is based on the detection of the various wavelength components. The presence of given fluorophores associated with the various analytes are detected based on their wavelength. When the particular fluorophore(s) associated with a particular analyte are determined to be present, that analyte is coded as being detected. **ddPCR Data Analysis** Droplet Digital PCR data can be viewed as a 1-D plot with each droplet from a sample plotted on the graph of fluorescence intensity vs. droplet number. In Figure 1.9, all positive droplets, those above the red threshold line, are scored as positive and each is assigned a value of 1. All negative droplets, those below the red threshold line, are scored as negative and each is assigned a value of 0.  **Fig. 1.9. Each droplet from a sample is plotted on the graph of fluorescence intensity vs. droplet number.**

Claims	Exemplary Disclosure of Accused Products
	Droplet Digital PCR data from a duplex experiment in which two targets are PCR amplified can also be viewed in a 2-D plot in which channel 1 fluorescence (FAM) is plotted against channel 2 fluorescence (HEX or VIC) for each droplet (Figure 1.10).  **Fig. 1.10. 2-D plot of droplet fluorescence.** **Droplet Reading** Following PCR amplification of the nucleic acid target in the droplets, place the PCR plate in a QX100 or QX200 Droplet Reader. The Droplet Reader and QuantaSoft Software count the PCR-positive and PCR-negative droplets to provide absolute quantification of target DNA. Droplet reading considerations are as follows: ▪ Before a run, the instrument can be set to interrogate droplets either in rows or columns ▪ Ensure there is enough Droplet Reader Oil in the instrument and the waste is empty before a run ▪ Each sample is processed individually and interrogated for both FAM and HEX (or VIC) fluorescence ▪ Data from 12,000–16,000 droplets are used in concentration calculations ▪ The reader measures fluorescence intensity of each droplet and detects the size and shape as droplets pass the detector; droplets are excluded if they do not meet quality metrics https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf. On information and belief, all infringing Bio-Rad ddPCR assays and kits are used in substantially the same way, regardless of

Claims	Exemplary Disclosure of Accused Products
	which device and software is used to perform the detection, because the ddPCR Workflow is substantially the same regardless of the specific assay or kit used. **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. BIO_CCODE00007427 at 7450; *see also* BIO_CCODE00006328 at 6351; BIO_CCODE00006554 at 6573. For all of Bio-Rad's infringing ddPCR assays and kits, the detection is unambiguous in that it detects the presence of the nucleic acid target sequences based on a coding scheme where the same detection cannot represent two or more analytes. For example: **Introduction** Droplet Digital polymerase chain reaction (ddPCR™) was developed to provide high-precision, absolute quantification of nucleic acid target sequences with wide-ranging applications for both research and clinical diagnostic applications. ddPCR measures absolute quantities by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. Droplet Digital PCR using Bio-Rad's QX100™ or QX200™ Droplet Digital PCR system overcomes the previous lack of scalable and practical technologies for digital PCR implementation. https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf.

Claims	Exemplary Disclosure of Accused Products

For all of Bio-Rad's infringing ddPCR assays and kits, the detection is performed in a single sample volume that is subsequently partitioned into multiple droplets.

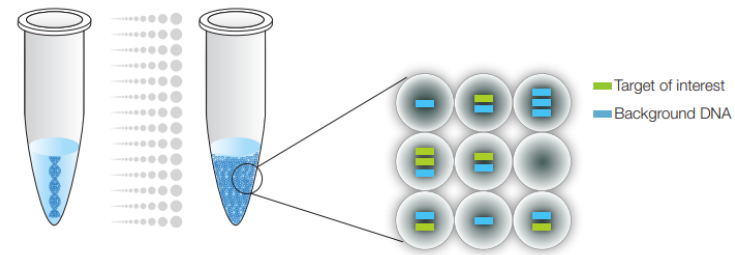


Fig. 1.3. In ddPCR, a single PCR sample is partitioned into 20,000 droplets.

https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf.

For the exemplary Accused products, M is always greater than F based on the analysis in [1a] and [1b], which is incorporated here by reference:

Assay / Kit	M	F
SARS-CoV-2 Kit	3	2
SARS-CoV-2 Wastewater Quantification Kit	3	2
MSI Kit (Assay 1)	3	2
MSI Kit (Assay 2)	3	2
The '3661 Assay	3	2
The '0842 Assay	5	4
The '4642 Assay	3	2

Examples of how this detection occurs for individual exemplary products are listed below.

SARS-CoV-2 Kit

In the SARS-CoV-2 Kit, the assay is capable of unambiguously detecting the presence or absence of each of N1, N2, and RP analytes, in any combination of presence or absence by using the coding scheme based on the detection of FAM and HEX as follows (1 = fluorophore is detected; 0 = not detected):

Analyte	FAM	HEX
N1	1	0
N2	1	1
RP	0	1

See BIO_CCODE00006914 at 6937, 6943. Based on the cumulative intensity measurements and as stated above, the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme.

SARS-CoV-2 Wastewater Quantification Kit

In the SARS-CoV-2 Wastewater Quantification Kit, the assay is capable of unambiguously detecting the presence or absence of each of N2, E, and MHV analytes, in any combination of presence or absence by using the coding scheme based on the detection of FAM and HEX as follows (1 = fluorophore is detected; 0 = not detected):

Analyte	FAM	HEX
N2	1	0
E	1	1
MHV	0	1

BIO_CCODE00006971 at 6979, 6985-6990. Based on the cumulative intensity measurements and as stated above, the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme.

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The MSI Kit

In the MSI Kit, Assay 1 of the MSI Kit is capable of unambiguously detect BAT25 mutant, BAT26 mutant, and BAT25 and BAT26 wild type; Assay 2 of the MSI Kit is capable of unambiguously detect NR21 mutant, NR24 mutant, and NR24 and NR21 wild type, both by using the coding scheme based on the detection of FAM and HEX as follows (1 = fluorophore is detected; 0 = not detected). Each assay is performed independently, such that Assay 1 analytes are in any combination of presence or absence, in a single sample volume, and Assay 2 analytes are any combination of presence or absence, in a single sample volume.

Assay 1 Analyte	FAM	HEX
BAT26 mutant	1	0
BAT25 mutant	0	1
BAT25 and BAT26 wild type	1	1

Assay 2 Analyte	FAM	HEX
NR24 mutant	1	0
NR21 mutant	0	1
NR24 and NR21 wild type	1	1

BIO_CCODE00006995 at 6997, 7019.

8. Label three 1.5 mL or 2.0 ml Eppendorf DNA LoBind Tubes, one for each assay. Prepare the reaction mixes as outlined in Table 6 (FFPE DNA samples) or Table 7 (cfDNA samples) for all three assays.

BIO-RAD EX.1012.022

Claims	Exemplary Disclosure of Accused Products

Table 6. Reaction Mix for FFPE DNA.

	Samples and Controls (reactions + overage) Volume (µl)				
Plates		**0.5**	**1**	**1.5**	**2**
Control Reactions		**4**	**4**	**8**	**8**
Sample Reactions		**12**	**28**	**40**	**56**
Total Reactions	**1**	**16**	**32**	**48**	**64**
Component	**1x**	**(16 + 2)x**	**(32 + 3)x**	**(48 + 4)x**	**(64 + 4)x**
Nuclease-free water	8.58	154.4	300.3	446.2	583.4
ddPCR Multiplex Supermix	5.5	99.0	192.5	286.0	374.0
ddPCR MSI Assay 1, 2 or 3	1.1	19.8	38.5	57.2	74.8
UDG*	0.22	4.0	7.7	11.4	15.0
Sample	6.6				
Total	22	277.2	539.0	800.8	1047.2

*UDG is needed for FFPE DNA samples only.

BIO_CCODE00006995 at 7004.

Based on the cumulative intensity measurements and as stated above, the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes in each Assay, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme.

<u>The ’3661 Assay</u>

In the dHsaEXD42003661 Assay, the assay is capable of unambiguously detecting the presence or absence of each of EGFR C797S G>C, EGFR C797S T>A, and EGFR C797S WT analytes, in any combination of presence or absence by using the coding scheme based on the detection of FAM and HEX as follows (1 = fluorophore is detected; 0 = not detected):

Analyte	FAM	HEX
EGFR C797S G>C	1	0

Claims	Exemplary Disclosure of Accused Products

EGFR C797S T>A	1	1
EGFR C797S WT	0	1

See BIO_CCODE00008545 at 8545, 8548. Based on the cumulative intensity measurements and as stated above, the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme.

The '0842 Assay

In the dHsaEXD41810842 Assay, the assay is capable of unambiguously detecting the presence or absence of the five analytes: T790M, L858R, C797S G>C, C797S T>A and C797 Wild Type in any combination of presence or absence by using the coding scheme based on the detection of FAM, HEX, Cy5, and Cy5.5 as follows (1 = fluorophore is detected; 0 = not detected):

Analyte	FAM	HEX	Cy5	Cy5.5
T790M	1	0	0	0
L858R	0	1	0	0
C797S G>C	0	0	1	0
C797S T>A	0	0	1	1
C797S Wild Type	0	0	0	1

See https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf at 1-4. Based on the cumulative intensity measurements and as stated above, the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 5 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme.

<table>
<tr><th>Claims</th><th>Exemplary Disclosure of Accused Products</th></tr>
<tr><td></td><td><u>The ’4642 Assay</u>

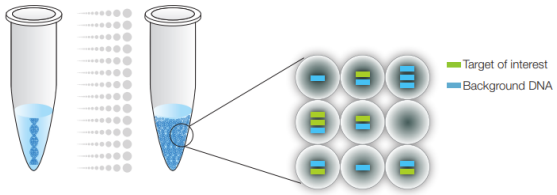
In the dEXD84844642 Assay, the assay is capable of unambiguously detecting the presence or absence of each of Influenza A, Influenza B, and RPP30 analytes, in any combination of presence or absence by using the coding scheme based on the detection of FAM and HEX as follows (1 = fluorophore is detected; 0 = not detected):

<table><tr><th>Analyte</th><th>FAM</th><th>HEX</th></tr><tr><td>Influenza A</td><td>1</td><td>0</td></tr><tr><td>Influenza B</td><td>1</td><td>1</td></tr><tr><td>Rpp30</td><td>0</td><td>1</td></tr></table>
BIO_CCODE00008542 at 8542-8544. Based on the cumulative intensity measurements and as stated above, the assay, as specified by the user guide, is capable of unambiguously detecting the presence or absence of 3 analytes, in any combination of presence or absence, as displayed by the 2D amplitude plots, cluster assignments, and the coding scheme.</td></tr>
<tr><td>[1d]

wherein the assay does not require the use of mass spectrometry or immobilization of said at least M analytes, and</td><td>For each infringing Bio Rad ddPCR Assay and Kit, use of such assay or kit does not require the use of any mass spectrometry or immobilization of any of the analytes. See generally https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf (describing ddPCR workflow without reference to using mass spectrometry or immobilization of analytes):</td></tr>
</table>

Claims	Exemplary Disclosure of Accused Products
	Overview Next-generation sequencing (NGS) systems are extremely sensitive to the quantity of library loaded in the sequencing run. Overloading frequently produces unusable data and underloading wastes reagents and time. Droplet Digital™ PCR (ddPCR™) complements NGS by offering accurate library concentration measurements and unique quality analyses that are not available with other methods. Standard methods for quantifying NGS libraries have disadvantages. Electrophoresis and spectrophotometry measure total nucleic acid concentrations, whereas optimal cluster density or template-to-bead ratio depends on the appropriate concentration of PCR-amplifiable DNA molecules. Incorrectly adapted products or adapter-adapter dimers cannot be distinguished easily from bona fide library fragments. These undesirable species can compete for binding sites on the flow cell, inhibit cluster formation, increase the likelihood of suboptimal loading, and reduce the total number of high-quality reads. These methods also have low sensitivity, consuming nanograms of precious samples, and are not suitable for high-throughput workflows. A more accurate method to quantify libraries before sequencing is required to maximize usage of sequencing platforms. ddPCR provides an absolute, standard-free method to measure library concentration and quality, while optimizing overall NGS performance. ddPCR makes very precise and accurate measurements of the library stock concentrations for flow-cell loading. The digital nature of droplet partitioning is conducive to accurate quantification by reducing competing PCR reactions. Likewise, the ddPCR Workflow identified in the QX200, QX600, and QX ONE systems describe using the assays and kits in ways that do not require either mass spectrometry or immobilization of analytes: **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets.

Claims	Exemplary Disclosure of Accused Products
	BIO_CCODE00007427 at 7450; *see also* BIO_CCODE00006328 at 6351; BIO_CCODE00006554 at 6573. Additionally, Bio-Rad's infringing ddPCR assays and kits do not force or preassign any clusters but instead permit direct quantification: **Direct Quantification with the Advanced Classification Method** The Advanced Classification Method analysis mode allows the user complete control of droplet identification, and does not force or preassign any clusters. This allows a higher degree of multiplexing through radial dye mixing. Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can click Undo to undo an applied well setting. Important: This mode is intended for experienced users of Droplet Digital PCR. BIO_CCODE00006328 at 6524 *See* also Element [1d.2] in Appendices A-1 and A-2; Element [1g] in Appendices B-1 and B-2.
[1e] **wherein the analytes are amplified in the single sample volume.**	The analytes are amplified in the single sample volume. For example, the single sample is partitioned into individual droplets:

Claims	Exemplary Disclosure of Accused Products
	Droplet Generation Before droplet generation, ddPCR reactions are prepared in a similar manner as real-time PCR reactions that use TaqMan hydrolysis probes labeled with FAM and HEX (or VIC) reporter fluorophores, or an intercalating dye such as EvaGreen®. ddPCR must be performed with the proprietary reagents developed specifically for droplet generation by Bio-Rad. Reagent mixes include the ddPCR Supermix for Probes and QX200™ ddPCR™ EvaGreen® Supermix to partition DNA, and the One-Step RT-ddPCR Advanced Kit for Probes. Samples are placed into a QX100 or QX200 Droplet Generator, which uses specially developed reagents and microfluidics to partition each sample into 20,000 nanoliter-sized droplets. As shown in Figure 1.3, target and background DNA are distributed randomly into the droplets during the partitioning process.  **Fig. 1.3. In ddPCR, a single PCR sample is partitioned into 20,000 droplets.** Droplet generation produces uniform droplets for the sample, enabling precise target quantification (Figure 1.4). https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf. Following droplet generation, the sample is amplified in a thermal cycler:

Claims	Exemplary Disclosure of Accused Products
	PCR Amplification Droplets are transferred to a 96-well plate for PCR in a thermal cycler. We recommend the C1000 Touch™ Thermal Cycler with 96–deep well reaction module for PCR (Figure 1.5). This high-performance thermal cycler has excellent temperature uniformity and settling across all 96 wells to help ensure successful PCR. Fig. 1.5. The C1000 Touch Thermal Cycler provides robust performance for ddPCR experiments. https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf. A materially similar process is followed regardless of which system (e.g., QX200, QX600, QXONE) is followed, although the QXONE has an integrated thermal cycler while the QX200 and QX600 rely on external thermal cyclers such as those identified above. **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. BIO_CCODE00007427 at 7450; *see also* BIO_CCODE00006328 at 6351; BIO_CCODE00006554 at 6573.

Claims	Exemplary Disclosure of Accused Products
2. The assay of claim 1, wherein each of said at least M analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.	Plaintiffs incorporate by reference the encoding analysis and cited evidence from claim elements [1a] and [1c]. As shown there, in each coding scheme for each exemplary accused assay or kit, each analyte for that assay/kit is encoded a 0 or 1 value for each of at least two component of the fluorescent signal (i.e. the fluorophore): **SARS-CoV-2 Kit:** **Analyte** / **FAM** / **HEX** **N1** 1 0 **N2** 1 1 **RP** 0 1 **SARS-CoV-2 Wastewater Quantification Kit:** **Analyte** / **FAM** / **HEX** **N2** 1 0 **E** 1 1 **MHV** 0 1 **MSI Kit:** **Assay 1 Analyte** / **FAM** / **HEX** **BAT26 mutant** 1 0 **BAT25 mutant** 0 1 **BAT25 and BAT26 wild type** 1 1 **Assay 2 Analyte** / **FAM** / **HEX** **NR24 mutant** 1 0 **NR21 mutant** 0 1

Claims	Exemplary Disclosure of Accused Products

NR24 and NR21 wild type	1	1

The ’3661 Assay

Analyte	FAM	HEX
EGFR C797S G>C	1	0
EGFR C797S T>A	1	1
EGFR C797S WT	0	1

The ’0842 Assay

Analyte	FAM	HEX	Cy5	Cy5.5
T790M	1	0	0	0
L858R	0	1	0	0
C797S G>C	0	0	1	0
C797S T>A	0	0	1	1
C797S Wild Type	0	0	0	1

The ’4642 Assay

Analyte	FAM	HEX
Influenza A	1	0
Influenza B	1	1
Rpp30	0	1

<table>
<tr><th>Claims</th><th>Exemplary Disclosure of Accused Products</th></tr>
<tr><td>3. The assay of claim 2, wherein said first value is an intensity or range of intensities.</td><td>Plaintiffs incorporate by reference the encoding analysis and cited evidence from claim elements [1a], [1c], and Claim 2. As shown there, for each infringing Bio-Rad assay or kit, the value is an intensity based on fluorescent signal and its wavelength.</td></tr>
<tr><td>4. The assay of claim 2, wherein said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.</td><td>Plaintiffs incorporate by reference the encoding analysis and cited evidence from claim elements [1a], [1c], and Claim 2. As shown there, the coding scheme for each infringing Bio-Rad ddPCR assay and kit, as shown by the exemplary assays and kits depicted in this chart, is made nondegenerate by enumerating every legitimate result that can be obtained from said coding scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy. For example,

The coding scheme of the Accused Products identify each legitimate result that is generate as shown below:

SARS-CoV-2 Kit:
<table>
<tr><th>Analyte</th><th>FAM</th><th>HEX</th></tr>
<tr><td>N1</td><td>1</td><td>0</td></tr>
<tr><td>N2</td><td>1</td><td>1</td></tr>
<tr><td>RP</td><td>0</td><td>1</td></tr>
</table></td></tr>
</table>

Claims	Exemplary Disclosure of Accused Products
	4. In the **Plate Editor** tab, select all the wells to be analyzed. With **Experiment Type** as **Direct Quantification (DQ)**; select **Probe Mix Triplex** under **Assay Information** and then click **Apply**. Enter Target information (Table 10) and click **Apply**. **Table 10. QuantaSoft Analysis Pro Target Information** Target Name | Signal Ch1 | Signal Ch2 N1 | FAM | None N2 | FAM | HEX RP | None | HEX BIO_CCODE00006914 at 6937 SARS-CoV-2 Wastewater Quantification Kit: **Analyte** | **FAM** | **HEX** **N2** | 1 | 0 **E** | 1 | 1 **MHV** | 0 | 1

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Claims	Exemplary Disclosure of Accused Products

Using QX Manager Software Standard Edition, set up a plate run, being sure to use the following plate and well parameters:

- Experiment Type: **Direct Quantification (DQ)**
- Sample Type: **Unknown** for samples, **NTC** for NTC, and **Pos Ctrl** for the RT-ddPCR control
- Supermix: **One-Step RT-ddPCR Advanced Kit for Probes**
- Assay Type: **Probe Mix Triplex**
- Targets:
  - N2 (Signal Ch1: **FAM**; Signal Ch2: **None**)
  - E (Signal Ch1: **FAM**; Signal Ch2: **HEX**)
  - MHV (Signal Ch1: **None**; Signal Ch2: **HEX**)

BIO_CCODE00006971 at 6979

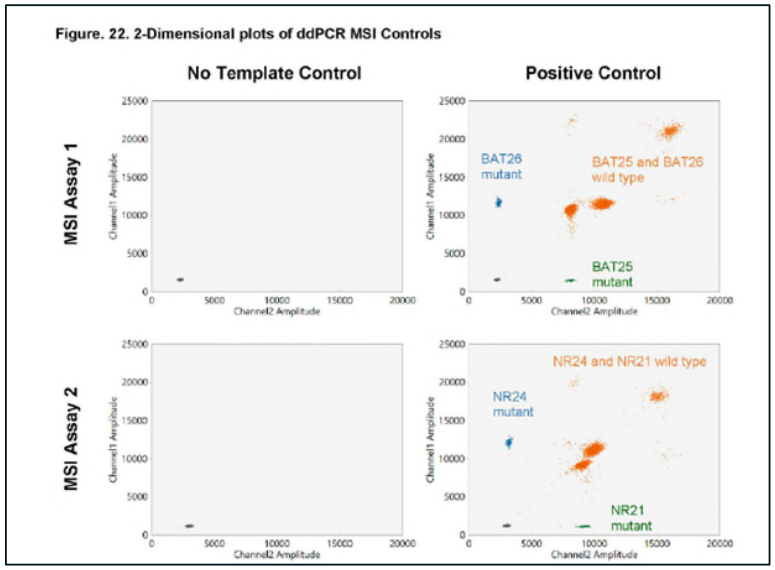
MSI Kit:

Assay 1 Analyte	FAM	HEX
BAT26 mutant	1	0
BAT25 mutant	0	1
BAT25 and BAT26 wild type	1	1

Assay 2 Analyte	FAM	HEX
NR24 mutant	1	0
NR21 mutant	0	1

Claims	Exemplary Disclosure of Accused Products

NR24 and NR21 wild type	1	1



BIO_CCODE00006995 at 7019

dHsaEXD42003661 Assay:

Analyte	FAM	HEX
EGFR C797S G>C	1	0
EGFR C797S T>A	1	1
EGFR C797S WT	0	1

Claims	Exemplary Disclosure of Accused Products

Targets Detected by the Assay

Variant Name	COSMIC ID	Fluorophore	Amplicon Length
EGFR C797S G>C	COSM5945664	FAM	98
EGFR C797S T>A	COSM6493937	FAM/HEX	98
EGFR C797S WT	N/A	HEX	98

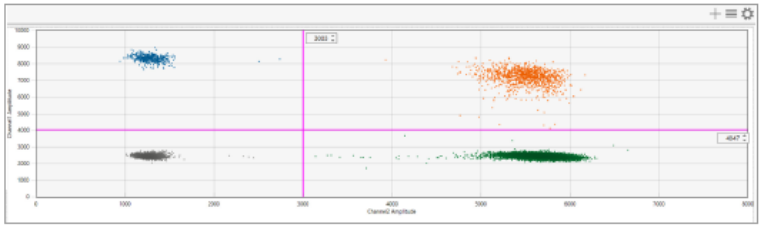
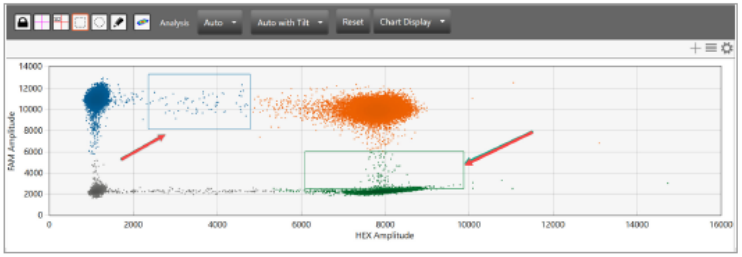
BIO_CCODE00008545 at 8545

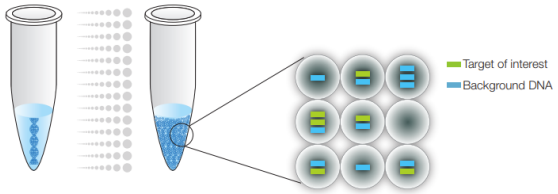
dHsaEXD41810842 Assay:

Analyte	FAM	HEM	Cy5	Cy5.5
T790M	1	0	0	0
L858R	0	1	0	0
C797S G>C	0	0	1	0
C797S T>A	0	0	1	1
C797S Wild Type	0	0	0	1

Claims	Exemplary Disclosure of Accused Products
	Expert Design Assay: Unique Assay ID dHsaEXD41810842 **Description** The EGFR Multiplex Assay is a 20X assay designed to discriminate EGFR; T790M, L858R, C797S T>A, and C797S G>C variants using Bio-Rad's QX ONE Droplet Digital PCR system. This assay should be validated prior to use by the end-user. **Targets Detected by the Assay** EGFR Variant Name | Cosmic ID | Fluorophore | Amplicon Length T790M | COSM6240 | FAM | 98 L858R | COSM6224 | HEX | 73 C797S G>C | COSM5945664 | Cy5 | 98 C797S T>A | COSM6493937 | Cy5/Cy5.5 | 98 C797 Wild type | N/A | Cy5.5 | 98 See dHsaEXD41810842 datasheet, available at https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf, page 1.

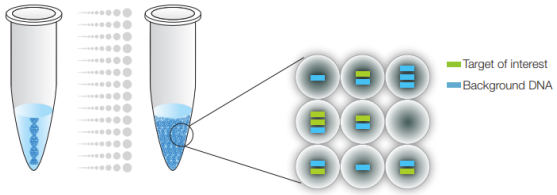
Claims	Exemplary Disclosure of Accused Products
	dEXD84844642: **Analyte** / **FAM** / **HEX** **Influenza A** 1 0 **Influenza B** 1 1 **Rpp30** 0 1 **Targets Detected by the Assay** Variant Name / ID / Fluorophore / Amplicon Length Influenza A / N/A / FAM / 106 Influenza B / N/A / FAM/HEX / 103 Rpp30 / ENST00000487998.5 / HEX / 103 BIO_CCODE00008542 at 8542 The user can manually adjust positive and negative sample thresholds to ensure correct quantification of nondegenerate result, and identify legitimate result that is degenerate and eliminate potential analyte code from the coding scheme to eliminate degeneracy. For example and as explained below, the manual thresholding tool allows for correct quantification of positive droplets (e.g., legitimate result that is non-degenerate) and negative droplets (e.g., legitimate result that is degenerate). The clusters can be grouped differently by adjusting the threshold lines to eliminate analyte code of at least one potential analyte from the coding scheme to eliminate degeneracy.

Claims	Exemplary Disclosure of Accused Products
	Note: Well data must meet certain quality metrics (minimum of 10,000 droplets) before QX Manager Software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets. ■ To adjust threshold lines, select a threshold line type ( ) and then click in the plot to apply crosshair lines. Use any of the following methods: □ Drag the lines horizontally or vertically in the plot area. □ Enter a new threshold value in the boxes at the end of each threshold line. □ Tap the up and down arrows to incrementally adjust the numbers.  ■ To adjust thresholds using the cluster option, select a cluster type ( ) and draw the chosen shape around the cluster of droplets to classify. 

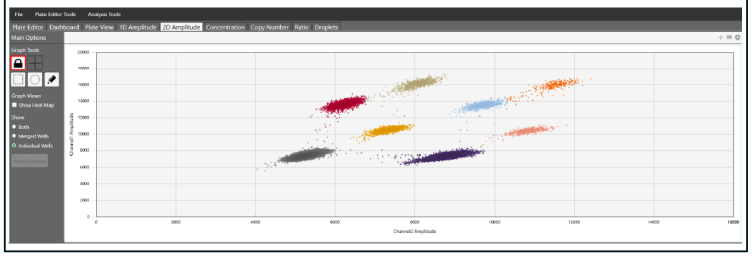
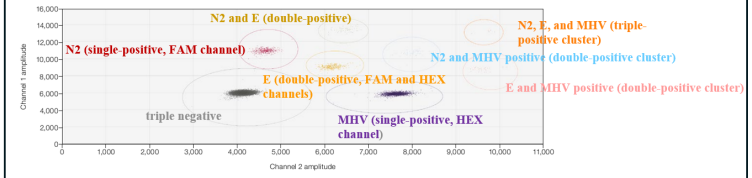
Claims	Exemplary Disclosure of Accused Products
	BIO_CCODE00006328 at 6462-6463
5. The assay of claim 1, wherein said detecting is performed with reagents comprising hybridization probes.	For each infringing Bio-Rad ddPCR assay and kit, reagents such as those identified above and in Appendices A-01, A-02, B-01, and B-02 are used with the assays: **Droplet Generation** Before droplet generation, ddPCR reactions are prepared in a similar manner as real-time PCR reactions that use TaqMan hydrolysis probes labeled with FAM and HEX (or VIC) reporter fluorophores, or an intercalating dye such as EvaGreen®. ddPCR must be performed with the proprietary reagents developed specifically for droplet generation by Bio-Rad. Reagent mixes include the ddPCR Supermix for Probes and QX200™ ddPCR™ EvaGreen® Supermix to partition DNA, and the One-Step RT-ddPCR Advanced Kit for Probes. Samples are placed into a QX100 or QX200 Droplet Generator, which uses specially developed reagents and microfluidics to partition each sample into 20,000 nanoliter-sized droplets. As shown in Figure 1.3, target and background DNA are distributed randomly into the droplets during the partitioning process.  **Fig. 1.3. In ddPCR, a single PCR sample is partitioned into 20,000 droplets.** Droplet generation produces uniform droplets for the sample, enabling precise target quantification (Figure 1.4). https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf. A materially similar process is followed regardless of which system (e.g., QX200, QX600, QXONE) is followed in that samples in a ddPCR process are prepared using primers, probes dye, and supermixes:

Claims	Exemplary Disclosure of Accused Products
	ddPCR Workflow The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. BIO_CCODE00007427 at 7450; *see also* BIO_CCODE00006328 at 6351; BIO_CCODE00006554 at 6573. **Table 8. ddPCR supermixes** **Supermix** / **Description** / **Catalog number** ddPCR EvaGreen® Supermix — 2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems — 1864033 2ml (2 x 1ml); 1864034 5ml (5 x 1ml); 1864035 25ml (25 x 1ml); 1864036 50ml (50 x 1ml) ddPCR Supermix for Probes (no dUTP) — 2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems — 1863023 2ml (2 x 1ml); 1863024 5ml (5 x 1ml); 1863025 25ml (25 x 1) ddPCR Supermix for Probes — 2x supermix, for use in sample preparation with the QX600 and QX200 ddPCR systems — 1863026 2ml (2 x 1ml); 1863010 5ml (5 x 1ml); 1863027 25ml (25 x 1ml); 1863028 50ml (50 x 1ml) ddPCR Multiplex Supermix — 4x supermix especially suited for probe-based detection of multiple targets in DNA samples using the QX600 and QX200 ddPCR systems — 12005909 1.2ml (2 x 0.6ml); 12005910 3 ml (5 x 0.6 ml); 12005911 12.5 ml (5 x 2.5 ml). BIO_CCODE00006328 at 6358.

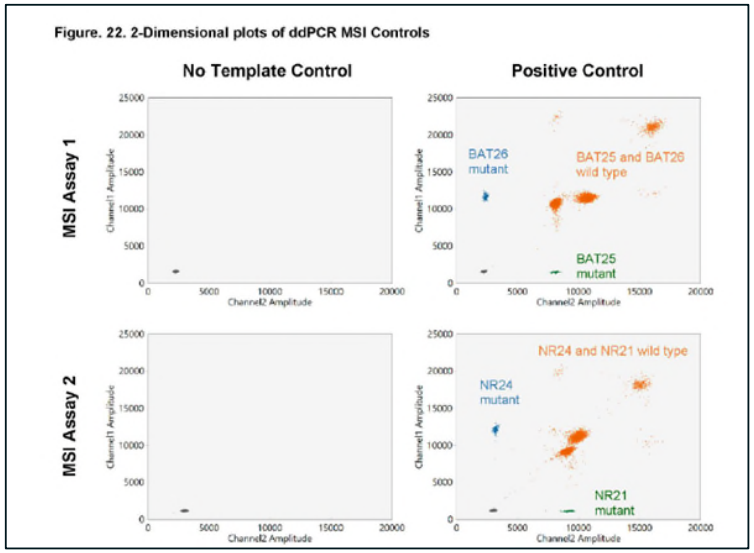
Claims	Exemplary Disclosure of Accused Products
	Section 2 **Kit Contents** The ddPCR Microsatellite Instability Kit contents are listed in Table 1. The kit contains sufficient reagents for 200 reactions. Upon receipt, store the kit in a constant temperature freezer at -15°C to -25°C. The kit reagents can be frozen and thawed up to three times. Repeated freezing and thawing of the kit reagents more than three times is not recommended. Reagents can be used until the expiration date indicated on the tube when stored properly. **Table 1. Kit contents (catalog #12015172).** Reagent | Material Number | Quantity ddPCR Multiplex Supermix (4x) | 12005915 | 2 ddPCR MSI Assay 1 (BAT25/BAT26) (20x) | 16009019 | 1 ddPCR MSI Assay 2 (NR21/NR24) (20x) | 16009020 | 1 ddPCR MSI Assay 3 (Mono27) (20x) | 16009029 | 1 ddPCR Positive Control | 16009021 | 1 Uracil-DNA Glycosylase (UDG) | 16009039 or 12017702 | 1 Nuclease-Free Water (no template control) | 8006877 | 2 BIOCCODE_00006995 at 6997. Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. BIO_CCODE00006175 at 6191. *See* also Element [1a.2] from Appendices A1 and A2; Claims 2, 3, 4, 9 from Appendices B1 and B2.

Claims	Exemplary Disclosure of Accused Products
6. The assay of claim 5, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.	Plaintiffs incorporate their analysis and cited evidence from Claim 5 here by reference. As discussed there, the infringing Bio-Rad ddPCR assays and kits are used with reagents that include one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores: **Droplet Generation** Before droplet generation, ddPCR reactions are prepared in a similar manner as real-time PCR reactions that use TaqMan hydrolysis probes labeled with FAM and HEX (or VIC) reporter fluorophores, or an intercalating dye such as EvaGreen®. ddPCR must be performed with the proprietary reagents developed specifically for droplet generation by Bio-Rad. Reagent mixes include the ddPCR Supermix for Probes and QX200™ ddPCR™ EvaGreen® Supermix to partition DNA, and the One-Step RT-ddPCR Advanced Kit for Probes. Samples are placed into a QX100 or QX200 Droplet Generator, which uses specially developed reagents and microfluidics to partition each sample into 20,000 nanoliter-sized droplets. As shown in Figure 1.3, target and background DNA are distributed randomly into the droplets during the partitioning process.  **Fig. 1.3. In ddPCR, a single PCR sample is partitioned into 20,000 droplets.** Droplet generation produces uniform droplets for the sample, enabling precise target quantification (Figure 1.4). https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf. A materially similar process is followed regardless of which system (e.g., QX200, QX600, QXONE) is followed in that samples in a ddPCR process are prepared using primers, probes dye, and supermixes:

Claims	Exemplary Disclosure of Accused Products
	ddPCR Workflow The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. BIO_CCODE00007427 at 7450; *see also* BIO_CCODE00006328 at 6351; BIO_CCODE00006554 at 6573. **Chapter 1 Introduction to Droplet Digital PCR** Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. BIO_CCODE00006175 at 6191. *See* also Element [1a.2] from Appendices A1 and A2; Claims 2, 3, 4, 9 from Appendices B1 and B2.
7. The assay of claim 5, wherein said fluorescence signal from said hybridization	The infringing Bio-Rad ddPCR assays and kits are used in systems such as the QX200, QX600, and QX ONE. Those systems include droplet readers that are used to construct chromatograms using the fluorescence signal from the hybridization probes. *See generally* A-01, A-02, B-01, and B-02 for exemplary disclosures of how the QX600 and QX ONE are used in this fashion.

Claims	Exemplary Disclosure of Accused Products
probes are used to construct a chromatogram.	The following are examples of chromatograms and other evidence generated for the various exemplary assays and kits discussed in this Appendix: **<u>SARS-CoV-2 Kit</u>** 16. Cluster assignments are applied upon selection. The final configuration will look like the image below.  BIO_CCODE00006914 at 6943. **<u>SARS-CoV-2 Wastewater Quantification Kit</u>** 1. Set the Assay Type for the plate to Triplex Probe Mix and make sure the targets are labeled/designated **N2** (FAM), **E** (FAM/HEX), and **MHV** (HEX). 2. In the 2D Amplitude tab, select all the wells to be analyzed.  Fig. 20. 2D plot with all correctly designated clusters. BIO_CCODE00006971 at 6985-6990.

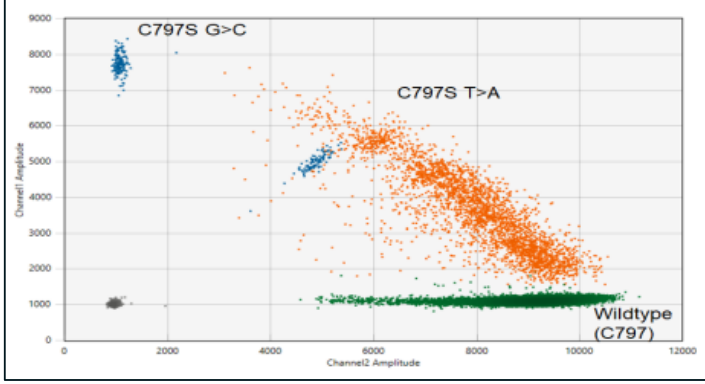
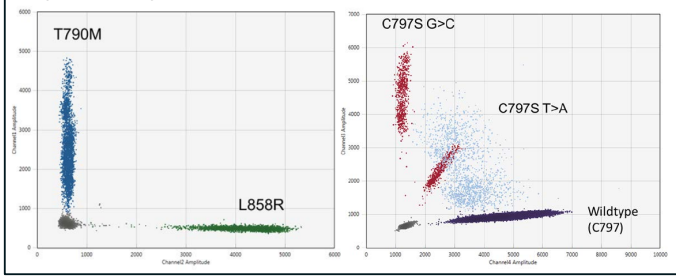
MSI Kit



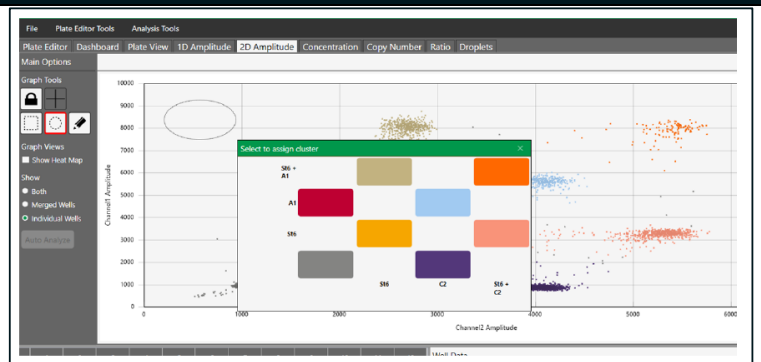
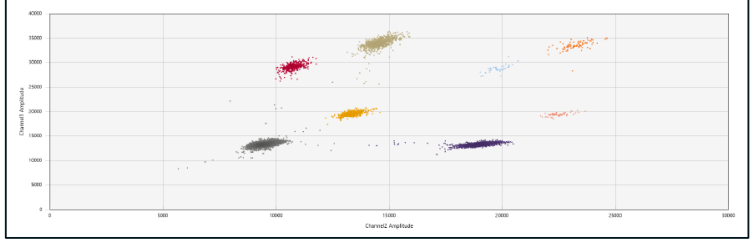
BIO_CCODE00006995 at 7019.

’3661 Assay:

BIO-RAD EX.1012.046

Claims	Exemplary Disclosure of Accused Products
	EGFR C797S G>C and *EGFR* C797S T>A overlayed to show relative position of droplet populations  BIO_CCODE00008545 at 8548. **<u>'0842 Assay:</u>** Figure 5: Example of all positives overlayed together to show relative positions of the positive droplets. 

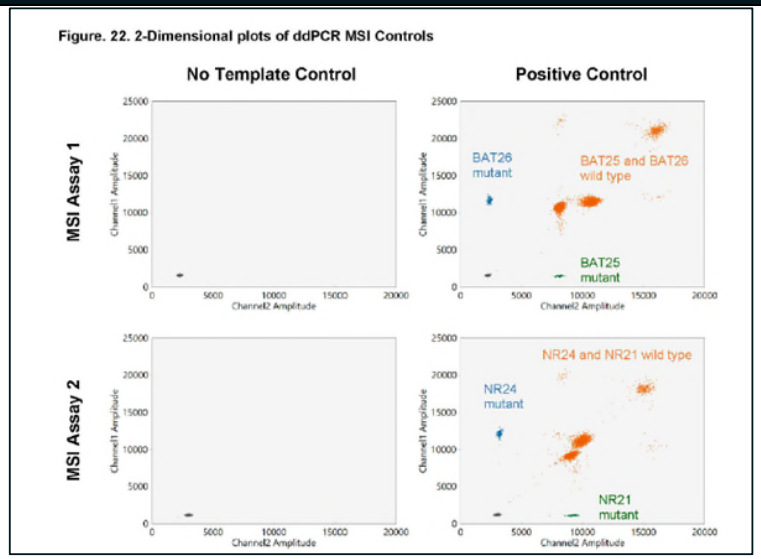
Claims	Exemplary Disclosure of Accused Products
	See dHsaEXD41810842 datasheet, available at https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf, page 3-4. **<u>'4642 Assay:</u>** **<u>Data Analysis</u>** After data file is finished running in QuantaSoft 1.7, use QuantaSoft Analysis Pro to analyze the 2D plots in the Probe Mix Triplex setting. In the Plate Editor tab choose Probe Mix Triplex in the Assay Information section and hit Apply. You can add all of your target names as follows: FAM-labeled assay (Influenza A), FAM/HEX-labeled assay (Influenza B) and HEX-labeled (RPP30). Next you will go to the 2D Amplitude tab and a clustering guide shown below can be used for simple clustering. The grid on this guide will also show which cluster has what targets. After clustering, the concentrations will be available in the table and in the concentration charts. BIO_CCODE00008542 at 8543.

Claims	Exemplary Disclosure of Accused Products
	 **Example Data**  BIO_CCODE00008542 at 8544.
8. The assay of claim 1, wherein said signal is generated during a polymerase chain reaction.	Each of the infringing Bio-Rad ddPCR assays and kits are used such that the signal is generated during a polymerase chain reaction. Each of the assays and kits use Droplet Digital PCR technology: The ddPCR Microsatellite Instability Kit detects deletions and insertions in five mononucleotide microsatellite markers (BAT25, BAT26, NR21, NR24, and Mono27) using Droplet Digital PCR technology, in plasma or tumor tissue, including formalin-fixed paraffin-embedded (FFPE) tissue from colorectal cancer patients and other potentially MSI-relevant cancers. These five markers have been classified as sensitive markers to size alterations and shifts in microsatellites in tumors with defective MMR systems (Bacher, et al. 2004). The ddPCR MSI Kit consists of three assays: assay 1 (BAT25 and BAT26), assay 2 (NR21 and NR24), and assay 3 (Mono27). This test does not assess the mutational status of MMR genes or whether any such mutations are somatic or germline.

Claims	Exemplary Disclosure of Accused Products
	BIO-CCODE00006995 at 6997. Droplet Digit PCR is a digital polymerase chain reaction method. It uses reagents and workflows similar to those used for most standard TaqMan probe-based assays: **Chapter 1 Introduction to Droplet Digital PCR** Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. BIO-CCODE00006175 at 6191. See also Claim 4 of Appendices A1 and A2. Claim 7 of Appendices B1 and B2.
9. The assay of claim 1, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal,	Plaintiffs incorporate by reference the analysis of claim elements [1a] and [1c]. As shown there, for each infringing Bio Rad ddPCR assay or kit, at least one anlalyte is encoded by at least one additional value, wherein that additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof. For example, In the SARS-CoV-2 Kit, the N2 Target is encoded by a positive signal of both FAM and HEX. 4. In the **Plate Editor** tab, select all the wells to be analyzed. With **Experiment Type** as **Direct Quantification (DQ)**; select **Probe Mix Triplex** under **Assay Information** and then click **Apply**. Enter Target information (Table 10) and click **Apply**. **Table 10. QuantaSoft Analysis Pro Target Information** | Target Name | Signal Ch1 | Signal Ch2 | | N1 | FAM | None | | N2 | FAM | HEX | | RP | None | HEX | BIO_CCODE00006914 at 6937.

Claims	Exemplary Disclosure of Accused Products
and combinations thereof.	In SARS-CoV-2 Wastewater Quantification Kit, the E Target is encoded by a positive signal of both FAM and HEX. Using QX Manager Software Standard Edition, set up a plate run, being sure to use the following plate and well parameters: ▪ Experiment Type: **Direct Quantification (DQ)** ▪ Sample Type: **Unknown** for samples, **NTC** for NTC, and **Pos Ctrl** for the RT-ddPCR control ▪ Supermix: **One-Step RT-ddPCR Advanced Kit for Probes** ▪ Assay Type: **Probe Mix Triplex** ▪ Targets: – N2 (Signal Ch1: **FAM**; Signal Ch2: **None**) – E (Signal Ch1: **FAM**; Signal Ch2: **HEX**) – MHV (Signal Ch1: **None**; Signal Ch2: **HEX**) BIO_CCODE00006971 at 6979 In the MSI Kit, on Assay 1 of the MSI Kit, BAT25 and BAT26 wild type is encoded as a positive of FAM fluorescent signal and a positive of a HEX signal. In Assay 2 of the MSI Kit, the NR24 wild type and NR21 wild type is encoded as a double-positive of both FAM and HEX signals.

Claims	Exemplary Disclosure of Accused Products



BIO_CCODE00006995 at 7019

The ’3661 Assay, the EGFR C797S T>A analyte is encoded by a positive signal of both FAM and HEX.

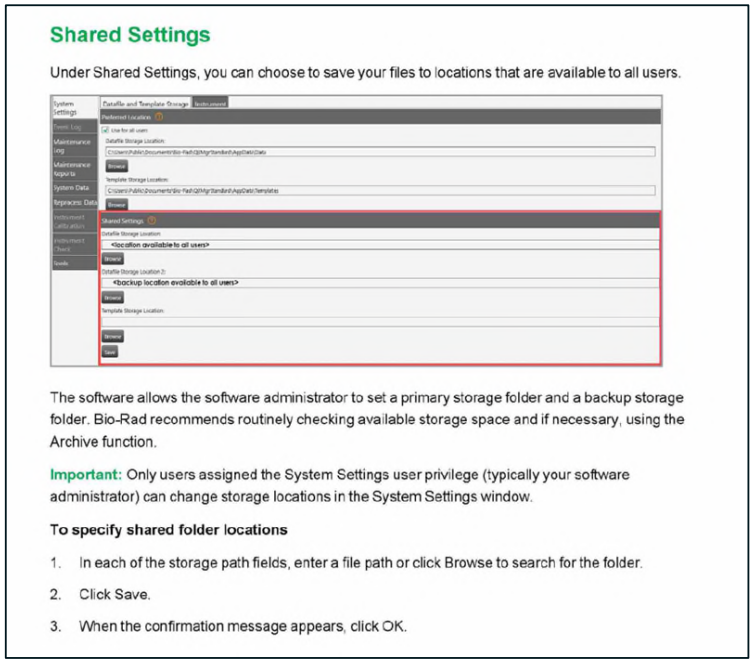
Targets Detected by the Assay

Variant Name	COSMIC ID	Fluorophore	Amplicon Length
EGFR C797S G>C	COSM5945664	FAM	98
EGFR C797S T>A	COSM6493937	FAM/HEX	98
EGFR C797S WT	N/A	HEX	98

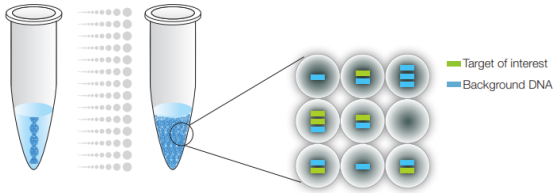
BIO_CCODE00008545 at 8545.

Claims	Exemplary Disclosure of Accused Products
	In the ’0842 Assay, C797S T>A is encoded as a positive signal of Cy5 and Cy5.5. **Expert Design Assay: Unique Assay ID dHsaEXD41810842** **Description** The EGFR Multiplex Assay is a 20X assay designed to discriminate EGFR; T790M, L858R, C797S T>A, and C797S G>C variants using Bio-Rad's QX ONE Droplet Digital PCR system. This assay should be validated prior to use by the end-user. **Targets Detected by the Assay** EGFR Variant Name | Cosmic ID | Fluorophore | Amplicon Length T790M | COSM6240 | FAM | 98 L858R | COSM6224 | HEX | 73 C797S G>C | COSM5945664 | Cy5 | 98 C797S T>A | COSM6493937 | Cy5/Cy5.5 | 98 C797 Wild type | N/A | Cy5.5 | 98 See dHsaEXD41810842 datasheet, available at https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf, page 1. In the ’4642 Assay, Influenza B is encoded as a positive of FAM signal and a positive of HEX signal. **Targets Detected by the Assay** Variant Name | ID | Fluorophore | Amplicon Length Influenza A | N/A | FAM | 106 Influenza B | N/A | FAM/HEX | 103 Rpp30 | ENST00000487998.5 | HEX | 103 BIO_CCODE00008542 at 8542.

Claims	Exemplary Disclosure of Accused Products
10. The assay of claim 1, wherein at least one of said analytes comprises a polynucleotide.	Plaintiffs incorporate by reference the analysis of claim elements [1a] and [1c]. As shown there, for each infringing Bio Rad ddPCR assay or kit, at least one of the analytes is a polynucleotide: • At least N1, N2, RP of SARS-CoV-2 Kit is a polynucleotide. *See* BIO_CCODE00006914 at 6937. • At least N2, E, MHV of SARS-CoV-2 Kit is a polynucleotide. *See* BIO_CCODE00006971 at 6979 • At least C797S WT of dHsaEXD42003661 Assay is a polynucleotide. *See* BIO_CCODE00008545 at 8545. • At least the T790M of dHsaEXD41810842 is a polynucleotide. See dHsaEXD41810842 datasheet, available at https://d1vjebusapavbw.cloudfront.net/digital-assays/static/assay/dHsaEXD41810842/dHsaEXD41810842_description.pdf, page 1. • At least influenza A and B of dEXD84844642 Assay are polynucleotides. *See* BIO_CCODE00008542 at 8542
11. The assay of claim 1, further comprising transmitting information concerning the presence or absence of at least one of said analytes through a computer network.	Plaintiffs incorporate by reference Claim 5 of Appendices A-01 and A-02 regarding how the QX600 and QX ONE systems can be used in connection with the infringing Bio-Rad ddPCR assays to transmit information concerning the presence or absence of at least one of said analytes through a computer network. The QX200 System can also be used in this regard. For example, it can share the saved files to predefined users.

Claims	Exemplary Disclosure of Accused Products
	 **Shared Settings** Under Shared Settings, you can choose to save your files to locations that are available to all users. The software allows the software administrator to set a primary storage folder and a backup storage folder. Bio-Rad recommends routinely checking available storage space and if necessary, using the Archive function. **Important:** Only users assigned the System Settings user privilege (typically your software administrator) can change storage locations in the System Settings window. **To specify shared folder locations** 1. In each of the storage path fields, enter a file path or click Browse to search for the folder. 2. Click Save. 3. When the confirmation message appears, click OK. BIO_CCODE00006328 at 6497.

Claims	Exemplary Disclosure of Accused Products
	Table 11 lists the available privileges, which can be assigned in any combination. **Table 11. User privileges** **Privilege** / **Purpose** Add and manage users — Add or remove users, set privileges, and change preferences. **Important:** Users who are assigned the Add/Manage Users privilege can create users and update privileges and preferences for any user. Only the superuser can remove this privilege from user accounts. Create new templates — Save a design for a plate or analysis report as a template. View data files (created by other users) — See run files that were created from runs executed by other users. Overwrite existing data file name — Save the file with a new file name. Files are always saved with the extension for the application you are using. For information, see Compatible File Types on page 48. **Note:** Users without this permission can open files and perform analysis but cannot create a new file. System settings — View logs, reprocess data with new calibration values, and change file locations. All users can view the file locations. **Important:** If your administrator selected the Preferred Locations checkbox in System Settings, individual user location preferences are overridden. Maintenance — View the event log and the system data settings. **Note:** All users can reprocess data, view system folder settings, as well as the maintenance log and maintenance reports. System Data — Move raw data from the computer to free up disk space. Access troubleshooting data. Manage Plate — Cancel a run while it is in process on the QX200 Droplet Reader. BIO_CCODE00006328 at 6371. As shown above, raw data may be moved across the network in order, for example, to free up disk space. Other transactions involving transmitting relevant data across a computer network is likely to come from additional discovery into the non-public operation of the source code.

Claims	Exemplary Disclosure of Accused Products
12. The assay of claim 1, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.	Plaintiffs incorporate their analysis from Claims 7 and 9 of Appendices A-01 and A-02 here. As discussed there, a thermal cycler can be a computer, whether it is standalone or incorporated into the system (as in the case of the QX ONE). One step of each of the infringing Bio Rad ddPCR assays is to prepare the sample with the necessary reagents and probes. Following this step, the next step of each ddPCR assay is the amplifiy the sample, which is performed on the thermal cycler / computer: **Droplet Generation** Before droplet generation, ddPCR reactions are prepared in a similar manner as real-time PCR reactions that use TaqMan hydrolysis probes labeled with FAM and HEX (or VIC) reporter fluorophores, or an intercalating dye such as EvaGreen®. ddPCR must be performed with the proprietary reagents developed specifically for droplet generation by Bio-Rad. Reagent mixes include the ddPCR Supermix for Probes and QX200™ ddPCR™ EvaGreen® Supermix to partition DNA, and the One-Step RT-ddPCR Advanced Kit for Probes. Samples are placed into a QX100 or QX200 Droplet Generator, which uses specially developed reagents and microfluidics to partition each sample into 20,000 nanoliter-sized droplets. As shown in Figure 1.3, target and background DNA are distributed randomly into the droplets during the partitioning process.  **Fig. 1.3. In ddPCR, a single PCR sample is partitioned into 20,000 droplets.** Droplet generation produces uniform droplets for the sample, enabling precise target quantification (Figure 1.4). https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf. Following droplet generation, the sample is amplified in a thermal cycler:

Claims	Exemplary Disclosure of Accused Products
	PCR Amplification Droplets are transferred to a 96-well plate for PCR in a thermal cycler. We recommend the C1000 Touch™ Thermal Cycler with 96–deep well reaction module for PCR (Figure 1.5). This high-performance thermal cycler has excellent temperature uniformity and settling across all 96 wells to help ensure successful PCR. Fig. 1.5. The C1000 Touch Thermal Cycler provides robust performance for ddPCR experiments. https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf. A materially similar process is followed regardless of which system (e.g., QX200, QX600, QXONE) is followed, although the QXONE has an integrated thermal cycler while the QX200 and QX600 rely on external thermal cyclers such as those identified above. **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. BIO_CCODE00007427 at 7450; *see also* BIO_CCODE00006328 at 6351; BIO_CCODE00006554 at 6573.

Claims	Exemplary Disclosure of Accused Products
	The said assay is performed using a computer and wherein said computer is connected to a thermal cycler. Additionally, some of the Accused Products are compatible with QX200 AutoDG Droplet Digital PCR System or the QX200 Droplet Digital PCR System. Both use a computer and the said computer is connected to a thermal cycler:

Claims	Exemplary Disclosure of Accused Products

Section 3

Required Equipment, Reagents, and Consumables

This kit is designed for use on the Bio-Rad QX200 AutoDG Droplet Digital PCR System or the QX200 Droplet Digital PCR System. Materials and instruments required but not supplied for use with the QX200 AutoDG Droplet Digital PCR System are listed in Table 2. Materials and instruments required but not supplied for use with the QX200 Droplet Digital PCR System are listed in Table 3. Adjustable Rainin and Eppendorf pipets can be used with the materials listed in Table 2 and Table 3.

Table 2. List of materials and instruments needed but not supplied with the ddPCR Microsatellite Instability Kit for use on the QX200 AutoDG Droplet Digital PCR System.

Description	Vendor	Catalog Number
Automated Droplet Generator	Bio-Rad	1864101
DG32 Automated Droplet Generator Cartridges	Bio-Rad	1864108
Pipet Tips for the AutoDG System	Bio-Rad	1864120
Pipet Tip Waste Bins for the AutoDG System	Bio-Rad	1864125
Automated Droplet Generation Oil for Probes	Bio-Rad	1864110
C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module	Bio-Rad	1851197
PX1 PCR Plate Sealer	Bio-Rad	1814000
PCR Plate Heat Seal	Bio-Rad	1814040
ddPCR 96-Well Plates	Bio-Rad	12001925
QX200 Droplet Reader	Bio-Rad	1864003
ddPCR Droplet Reader Oil	Bio-Rad	1863004
ddPCR Buffer Control for Probes (2x)	Bio-Rad	1863052
QX Manager Software Premium Edition, v2.1 or higher*	Bio-Rad	12018108
MSI Assay Protocol File, MSI10_FFPE_QR.apfpack**	Bio-Rad	N/A
MSI Assay Protocol File, MSI10_FFPE.apfpack**	Bio-Rad	N/A
MSI Assay Protocol File, MSI10_Plasma_QR.apfpack**	Bio-Rad	N/A
MSI Assay Protocol File, MSI10_Plasma.apfpack**	Bio-Rad	N/A
Vortexer	Any	N/A
Microcentrifuge	Any	N/A
Low TE Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0)	Any	N/A

* If not already installed, go to bio-rad.com/QXSoftware to download and install the latest QX Manager Software.

** Go to bio-rad.com/ddPCR-MSI to download the ddPCR MSI Assay Protocol Files.

Claims	Exemplary Disclosure of Accused Products
	Table 3. List of materials and instruments needed but not supplied with the ddPCR Microsatellite Instability Kit for use on the QX200 Droplet Digital PCR System. Description | Vendor | Catalog Number QX200 Droplet Generator | Bio-Rad | 1864002 DG8 Cartridges for QX200 Droplet Generator | Bio-Rad | 1864008 DG8 Gaskets for QX200 Droplet Generator | Bio-Rad | 1863009 DG8 Cartridge Holder | Bio-Rad | 1863051 Droplet Generation Oil for Probes | Bio-Rad | 1863005 C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module | Bio-Rad | 1851197 PX1 PCR Plate Sealer | Bio-Rad | 1814000 PCR Plate Heat Seal, foil, pierceable | Bio-Rad | 1814040 QX200 Droplet Reader | Bio-Rad | 1864003 ddPCR 96-Well Plates | Bio-Rad | 12001925 ddPCR Droplet Reader Oil | Bio-Rad | 1863004 ddPCR Buffer Control for Probes (2x) | Bio-Rad | 1863052 QX Manager Software Premium Edition, v2.1 or higher* | Bio-Rad | 12018108 MSI Assay Protocol File, MSI10_FFPE_QR.apfpack ** | Bio-Rad | N/A MSI Assay Protocol File, MSI10_FFPE.apfpack** | Bio-Rad | N/A MSI Assay Protocol File, MSI10_Plasma_QR.apfpack** | Bio-Rad | N/A MSI Assay Protocol File, MSI10_Plasma.apfpack** | Bio-Rad | N/A Vortexer | Any | N/A Microcentrifuge | Any | N/A Low TE Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) | Any | N/A * If not already installed, go to bio-rad.com/QXSoftware to download and install the latest QX Manager Software. ** Go to bio-rad.com/ddPCR-MSI to download the ddPCR MSI Assay Protocol Files. BIO_CCODE00006995 at 6998-BIO_CCODE00006999

Claims	Exemplary Disclosure of Accused Products

Section 5

Protocol

It is strongly recommended to read the entire protocol before starting the test. The ddPCR MSI protocol consists of the following steps:

Step	Description	Instrument/Tool	Hands-on Time	Instrument Time
1	DNA extraction (FFPE or plasma)	Variable	Variable	Variable
2	Plate setup and droplet generation	or	<40 min	~45 min/plate
3	PCR amplification		<5 min	~150 min (~120 min for thermal cycling and ~30 min for cooling)
4	Droplet reading		<5 min	~120 min/plate

7 ddPCR™ Microsatellite Instability Kit

BIO_CCODE00006995 at 7001.

Thermal Cycling

1. Transfer the plate to a thermal cycler and begin PCR amplification within 30 minutes of sealing the plate, using the thermal cycling conditions in Table 8.

   Note: It is important to use a thermal cycler with an adjustable ramp rate. The ramp rate should be set to 2°C/sec.

Claims	Exemplary Disclosure of Accused Products
	Table 8. Thermal cycling conditions. Temperature, °C: 95; Time: 10 min; Cycles: 1; Ramp Rate, °C/s: 2 Temperature, °C: 94; Time: 30 sec; Cycles: 40 Temperature, °C: 55; Time: 1 min Temperature, °C: 98; Time: 10 min; Cycles: 1 Temperature, °C: 4; Time: Hold; Cycles: 1 2. Upon completion of the thermal cycling run, let the plate hold at 4°C for 30 min or until the lid temperature cools to 37°C. **Note:** The plate may be stored at 2°C to 8°C overnight before analysis on the QX200 Droplet Reader. BIO_CCODE00006995 at 7005- BIO_CCODE00007006 **Thermal Cyclers and Plate Sealer** **Table 47. Thermal cyclers and plate sealer** Product: C1000 Touch Thermal Cycler with 96-Deepwell Reaction Module; Description: Includes the C1000 Touch Thermal Cycler chassis, 96-deepwell reaction module, USB flash drive; Catalog Number: 1851197 Product: PTC Tempo Deepwell Thermal Cycler; Description: Includes the thermal cycler, power cord, USB cable, and Ethernet cable; Catalog Number: 12015392 Product: PX1 PCR Plate Sealer; Description: PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord; Catalog Number: 1814000 Product: Pierceable Foil Heat Seal; Description: Heat seal for the PX1 PCR Plate Sealer; Catalog Number: 1814040 BIO_CCODE00006328 at 6545 The QX ONE also is a computer and has an integrated thermal cycler. *See* Claim 9 of Appendix A-02.

Claims	Exemplary Disclosure of Accused Products
13. The assay of claim 1, wherein said coding scheme is innately nondegenerate or non-degenerate by construction.	The said coding scheme is innately nondegenerate or non-degenerate by construction. For example, *See* Elements [1a], [1c] Claims 2 and 4.
[14-PRE] **14. A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:**	*See* Claim 1.
[14a] **a. each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme,**	Each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy. For example, *See* Claims [1a], [1c], 2, 4, 13.

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Claims	Exemplary Disclosure of Accused Products
wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and	
[14b] **b. each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value;**	Each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value. For example, *See* Claims [1a], [1b], [1c], 5-7.
[14c] **wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single**	Wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence. *See* Claim [1c], [1d], and [1e]. Plaintiffs note that there is a printing error in the publication of the ’921 patent. Based on the Claims on October 10, 2023, p. 34, this claim limitation should be “wherein the analytes are amplified in the single sample volume.”

Claims	Exemplary Disclosure of Accused Products
sample volume, in any combination of presence or absence, and wherein the kit does not require the use of mass spectrometry or immobilization of said M analytes, and wherein the analytes are [amplified] in the single sample volume.	*See* Claim 1.
15. The kit of claim 14, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F.	Wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F. *See* Claim [1b], [1c].

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Claims	Exemplary Disclosure of Accused Products
16. The kit of claim 14, wherein said analyte-specific reagents comprise a plurality of hybridization probes.	Wherein said analyte-specific reagents comprise a plurality of hybridization probes. For example, *See* Claims 5-7.
17. The kit of claim 16, wherein said plurality of hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes.	Wherein said plurality of hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes. *See* Claims 5-7.
19. The kit of claim 14, wherein the analyte-specific reagents are each present at a different amount in the kit.	Wherein the analyte-specific reagents are each present at a different amount in the kit. For example, The analyte-specific reagents, target primers/probe can be in 900 nM/250 nM concentration.

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Claims	Exemplary Disclosure of Accused Products
	Table 1. Preparation of the reaction mix. | Component | Volume per Reaction, µl | Final Concentration | | 2x ddPCR Supermix for Probes | 10 | 1x | | 20x target primers/probe (FAM, Cy5, or ROX) | 1 | 900 nM/250 nM | | 20x target primers/probe (HEX/VIC, Cy5.5, or ATTO 590) | 1 | 900 nM/250 nM | | Diluted restriction enzyme (see DNA Digestion section) | 1 | Variable | | Sample | Variable | Up to 330 ng* | | RNase-/DNase-free water | Variable | – | | **Total volume**** | **20** | **–** | * Sample concentrations >66 ng per reaction and certain applications may require restriction digestion for optimal target detection. If digestion is not required, prepare the ddPCR reaction mix without the diluted restriction enzyme. ** For the Automated Droplet Generator, prepare 22 µl per reaction. BIO_CCODE00007875 at 7875. *See also*, *e.g.*, other reagent and assay instructions showing different amounts of specific reagents, such as BIO_CCODE00007877, BIO_CCODE00007881, BIO_CCODE00007879, BIO_CCODE00006173, BIO_CCODE00006223, BIO_CCODE00006227, BIO_CCODE00006313, BIO_CCODE00006315, BIO_CCODE00006316, BIO_CCODE00006324, BIO_CCODE00006969, BIO_CCODE00006995, BIO_CCODE00007033, BIO_CCODE00007037, BIO_CCODE00007875, BIO_CCODE00007877, BIO_CCODE00007879, BIO_CCODE00007881, BIO_CCODE00008188, BIO_CCODE00008198.

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