EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
ID-FISH Plasmodium Genus Test Kit
ID-FISH Plasmodium falciparum and P. vivax Combo Test Kit
DECISION SUMMARY

A. DEN Number:

DEN160025

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation for the ID-FISH Plasmodium Genus Test Kit and the ID-FISH Plasmodium falciparum and P. vivax Combo Test Kit

C. Measurands:

Ribosomal RNA sequences of *Plasmodium falciparum, Plasmodium vivax,* and other *Plasmodium* species

D. Type of Test:

Fluorescence In Situ Hybridization (FISH) assay using fluorescently labeled DNA probes

E. Applicant:

ID-FISH Technology, Inc.

F. Proprietary and Established Names:

ID-FISH Plasmodium Genus Test Kit (PlasG)

ID-FISH Plasmodium falciparum and P. vivax Combo Test Kit (PlasFV)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3367

2. Classification:

   Class II (Special Controls)

3. Product code(s):

   PYN

4. Panel:

   83- Microbiology

H. Indications for Use:

1. Indications for use:

   ID-FISH Plasmodium Genus Test Kit (PlasG) and ID-FISH Plasmodium falciparum and P. vivax Combo Test Kit (PlasFV) are intended for *in vitro* diagnostic use in the clinical laboratory for detection of *Plasmodium* species in human venous whole blood (EDTA) samples from patients suspected of *Plasmodium* infection. The test kits are intended to aid in the diagnosis of malaria and to aid in the differential diagnosis of *P. falciparum* and *P. vivax* infection. The test kits should be used only on samples from patients with a clinical history, signs and symptoms consistent with malaria, and are not intended as a screen for asymptomatic patients.

   The **ID-FISH Plasmodium Genus Test Kit** is a qualitative test for detection of malaria parasites in blood smears. Positive results should be supplemented with the *Plasmodium* species specific test kit, **ID-FISH Plasmodium falciparum and P. vivax Combo Test Kit** for identification and differentiation of *Plasmodium falciparum* and *Plasmodium vivax*. The results of these test kits should be used in conjunction with other diagnostic test results. Clinical performance has not been established for *P. ovale*, *P. malariae*, or *P. knowlesi*.

2. Special conditions for use statement(s):

   For in vitro diagnostic use only
   For prescription use only
   For professional use only
   The product has not been validated with specimens other than venous whole blood (EDTA)

3. Special instrument requirements:

   Fluorescence microscope equipped with the following:
   - Mercury arc lamp or LED light powered illumination
   - Dual band filter set compatible with ID-FISH hybridization probes
   - 100x oil objective

I. Device Description:

The ID-FISH Plasmodium Genus Test Kit (PlasG) and ID-FISH Plasmodium falciparum and

P. vivax Combo Test Kit (PlasFV) are fluorescence *in situ* hybridization (FISH) assays to detect *Plasmodium spp.* or *P. falciparum* or *P. vivax* parasites in thin film blood smears prepared from EDTA venous whole blood samples.

Materials provided:

ID-FISH Plasmodium Genus Test Kit

- Plasmodium Genus Hyb A
- Plasmodium Hyb B
- 5x Plasmodium Wash Buffer
- 10x Plasmodium Rinse Buffer
- Plasmodium Counterstain

ID-FISH Plasmodium falciparum and P. vivax Combo Test Kit

- Plasmodium falciparum / P. vivax Hyb A
- Plasmodium Hyb B
- 5x Plasmodium Wash Buffer
- 10x Plasmodium Rinse Buffer
- Plasmodium Counterstain

Materials not provided, but available for separate purchase:

- SPR – Smear Preparation Reagent
- Dual Band Filter Set specific to ID-FISH hybridization probes

Materials required but not provided:

- Fluorescence microscope with 100x oil objective
- 37±1º C Incubator
- Negative Control Smears (made with non-malarial EDTA blood less than four days old and SPR)
- Positive Control Smears (made with EDTA blood infected with *P.f.* or *P.v.* parasites less than four days old with SPR)

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

Not applicable

K. Test Principle:

Fluorescent *in situ* hybridization (FISH) is a microscopic technique in which a DNA probe is labeled with a fluorescent dye and then hybridized on a slide with a target region of nucleic acid of the pathogen in a clinical sample. Venous whole blood from a patient (EDTA preserved) suspected of malaria is mixed with a fixative (smear preparation reagent), smeared onto a glass slide in a thin film, air-dried, and preserved with methanol. The smear

is then hybridized with a fluorescently-labeled DNA probe complementary to the rRNA of *Plasmodium* genus, *P. falciparum,* or *P. vivax,* depending on the probe used. *Plasmodium* parasites are visible in the sample when viewed with a fluorescence microscope equipped with the appropriate filters, and non-*Plasmodium* organisms do not produce a signal under the filters. *Plasmodium* species, if present, will appear green with the *Plasmodium* genus probe in the PlasG test kit. *P. falciparum* will appear green with the *P. falciparum* probe, and *P. vivax* will appear orange with the *P. vivax* probe in the PlasFV test kit. Ribosomal RNA is present in the cytoplasm of the parasite and is visible within the host red blood cell so that the entire parasite will give a fluorescent signal. Both sexual and asexual forms of *Plasmodium spp.*, *P. falciparum* and *P. vivax* parasites are labelled by the respective PlasG and PlasFV test kit probes. Samples from patients who are receiving anti-malarial drug treatment may have negative FISH results, while Giemsa microscopy and PCR may remain positive for longer periods of time after treatment.

L. Performance Characteristics:

1. Analytical performance:

a. Reproducibility

A study was conducted to evaluate the reproducibility of the ID-FISH Plasmodium Genus Test Kit (PlasG) and the ID-FISH Plasmodium falciparum and P. vivax Combo Test Kit (PlasFV). A panel of five samples was contrived in human EDTA whole blood at the following parasite concentrations:

- Moderate positive *P. falciparum* – 480 parasites/µL
- Moderate positive *P. vivax* – 440 parasites/µL
- Low positive *P. falciparum* – 156 parasites/µL
- Low positive *P. vivax* – 160 parasites/µL
- Negative – 0 parasites/µL

One hundred and eighty (180) replicate slides containing two smears per slide were prepared from each sample at two separate sites (900 slides total). The randomized and blinded panel was distributed to three sites (300 slides per site) and tested over five non-consecutive days, with two operators per site performing two runs per day. For each run, each blinded panel member was tested in triplicate with both the PlasG and PlasFV test kits using two smears per slide (3 replicates x 2 operators x 2 runs x 5 days x 3 sites = 180 observations per kit).

Of the 900 slides, three smears (one negative smear and two low positive *P. falciparum* smears) fell off the slides at one site and so could not be read (3/900 = 0.3%). These smears were excluded from the analysis.

For the PlasG test kit, one negative smear gave a false positive result in one run for a negative agreement of 99.4% (178/179). There was 100% agreement with the expected result with the PlasG test kit for all other panel members across runs, days, and sites. See Table 1.

For the PlasFV test kit, one false positive result was observed for one negative smear (note: the same negative smear that also gave a false positive result with the PlasG test kit) for a negative agreement of 99.4% (178/179). One low positive *P. falciparum*

sample produced a false negative result in one run for an agreement of 99.4% (177/178). There was 100% agreement with the expected *P. falciparum, P. vivax,* or negative results with the PlasFV test kit for all other panel members across operators, runs, days, and sites. See Table 2.

Table 1. PlasG test kit reproducibility

Sample	Agreement with expected result (%)						Overall	95% Confidence Interval
	Site 1		Site 2		Site 3			
	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2		
Negative	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	28[a]/29[b] (96.6)	178[a]/179[b] (99.4)	96.9 – 99.9%
Low *P.f.*	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	28/28[b] (100)	178/178[b] (100)	97.9 – 100%
Mod. *P.f.*	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	180/180 (100)	97.9 – 100%
Low *P.v.*	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	180/180 (100)	97.9 – 100%
Mod. *P.v.*	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	180/180 (100)	97.9 – 100%

[a] One negative smear gave a positive result for Operator 2 at Site 3.
[b] Smear(s) fell off.

Table 2. PlasFV test kit reproducibility

Sample	Agreement with expected result (%)						Overall	95% Confidence Interval
	Site 1		Site 2		Site 3			
	Op 1	Op 2	Op 1	Op 2	Op 1	Op 2		
Negative	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	28[a]/29[b] (96.6)	178[a]/179[b] (99.4)	96.9 – 99.9%
Low P.f.	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	27[c]/28[b] (96.4)	177[c]/178[b] (99.4)	96.9 – 99.9%
Mod. P.f.	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	180/180 (100)	97.9 – 100%
Low P.v.	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	180/180 (100)	97.9 – 100%
Mod. P.v	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	30/30 (100)	180/180 (100)	97.9 – 100%

[a] One negative smear gave a false positive result for Operator 2 at Site 3.
[b] Smear(s) fell off.
[c] One low positive *P.f.* smear gave a false negative result for Operator 2 at Site 3.

b. *Linearity/assay Reportable Range:*

Not Applicable

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

Recommended External Controls:

Control material should be tested in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. To monitor the

assay, reagent performance and day-to-day variation, positive controls for *P. falciparum* or *P. vivax* along with a negative control (non-malarial blood) must be tested with each run. Negative controls can be prepared from EDTA whole blood collected from normal subjects. Positive slides can be prepared from previously positive patient blood samples. Control slides should be prepared in the same manner as patient samples and they are stable for five years if stored in a dry place at room temperature. During the clinical testing conducted at three sites, external positive and negative FISH controls were tested for each run as per kit instructions, and no control failures were observed for the 61 days of non-consecutive clinical testing across all sites.

d. Detection Limit:

The analytical limit of detection (LoD) was estimated for the PlasG and PlasFV test kits using well defined clinical samples serially diluted in EDTA whole blood. A *P. falciparum* positive sample with a neat parasitemia of 18,375 parasites per microliter and a *P. vivax* positive sample with a neat parasitemia of 5,040 parasites per microliter were tested. The *P. falciparum* sample was serially diluted as follows: 1:16, 1:32, 1:64, 1:128, and 1:256. The *P. vivax* sample was serially diluted as follows: 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320. Triplicate smears were prepared from each dilution and tested with both kits, and the highest dilution (i.e. lowest parasite concentration) which produced a positive result for all three replicates was considered the preliminary LoD. The LoD was confirmed by testing an additional 20 smears prepared from blood containing parasites at this concentration to demonstrate a detection rate of greater than or equal to 95%. The LoD for each kit is summarized in Table 3.

Table 3. Limit of detection (parasites/µL)

	P. falciparum	***P. vivax***
PlasG FISH	143	126
PlasFV FISH	143	126

e. Analytical Reactivity (Inclusivity):

The inclusivity of the PlasG and PlasFV test kits was evaluated with an additional 11 blood samples positive for *Plasmodium* species. Ten human clinical blood samples from different geographic regions were tested including four *P. falciparum*, two *P. vivax*, two *P. ovale*, and two *P. malariae* positive samples. One monkey blood *P. knowlesi* positive sample was also tested. All *Plasmodium* positive samples were detected by the PlasG test kit. All *P. falciparum* and *P. vivax* positive samples were correctly identified by the respective probes in the PlasFV test kit.

f. Analytical Specificity:

Cross reactivity

The cross reactivity of the PlasG and PlasFV test kits was evaluated with a panel of 29 pathogenic bacteria, parasites, and viruses that may be found in human blood

samples. Microorganisms were spiked into whole blood samples and then blood smear slides were prepared according to the kit instructions. Except as noted, all parasites and bacteria were tested at concentrations greater than 10^6 organisms per milliliter and viruses were tested at greater than 10^5 PFU or copies per milliliter. For the PlasFV test kit, off-target *Plasmodium* species were also evaluated (e.g., non-*P. vivax* species to evaluate the *P. vivax* probe cross reactivity). See Table 4. No off-target organism produced a positive result with PlasG or PlasFV test kits.

Table 4. Cross reactivity panel

Microorganisms
Anaplasma phagocytophilum
Babesia duncani
Babesia microti
Bartonella henselae
Borrelia burgdorferi
Bwamba Fever Virus
Chikungunya virus
Cytomegalovirus (CMV)
Dengue virus
Ehrlichia chaffeensis
Epstein-Barr virus (EBV)
Hepatitis B
HIV-1[a]
Influenza A
Influenza B
Leishmania major (amastigotes)
Leishmania major (promastigotes)
Leptospira interrogans
Rickettsia rickettsii
Rickettsia typhi
Trypanosoma cruzi
West Nile virus
Yellow Fever virus
Plasmodium berghei [a,b]
Plasmodium falciparum [a,b]
Plasmodium malariae [a,b]
Plasmodium knowlesi [a,b]
Plasmodium ovale [a,b]
Plasmodium vivax [a,b]

[a] Tested at > 10^4 organisms/mL

[b] All on-target *Plasmodium* species were detected with the expected PlasG and PlasFV test kit probes.

Additionally, cross reactivity was evaluated with a panel of 76 blood samples from patients with non-malarial medical conditions that were processed as blood smears according to the kit instructions. These included 42 blood samples from patients with positive antibody test results for one or more of the following tick-borne diseases: *B. burgdorferi, Babesia spp, B. henselae, A. phagocytophilum, E. chaffeensis,* and *Rickettsia spp.*; 20 samples from patients with positive antinuclear antibody test results; four samples from patients with hepatitis B infection; and ten blood samples spiked with plasma from ten patients with HIV1 infection. No false positive results were observed with the PlasG or PlasFV test kits.

Interfering Substances

The performances of PlasG and PlasFV test kits were evaluated in the presence of potentially interfering exogenous and endogenous substances that may occur in blood. Human whole blood samples spiked with *P. falciparum, P. vivax,* or unspiked controls were prepared as smears according to the kit instructions and tested in the presence of potential interferents at the concentrations shown in Tables 5 and 6. Each *Plasmodium* positive sample was detected by the expected PlasG and PlasFV test kit probes in the presence of the spiked substances. No false positive results were observed in the uninfected control blood samples in the presence of the spiked substances.

Table 5. Endogenous potential interfering substances

Substance	Concentration
Triglycerides	37 mmol/L
Hemoglobin	2 g/L
Bilirubin	342 µmol/L
Serum albumin	60 g/L

Table 6. Exogenous potential interfering substances

Substance	Concentration
Acetaminophen	1 mg/mL
Aspirin	1 mg/mL
Ibuprofen	1 mg/mL
Artesunate	1 mg/mL
Chloroquine	1 mg/mL
Doxycycline	1 mg/mL
Primaquine	1 mg/mL
Quinine	1 mg/mL
Sulfadoxine	1 mg/mL
Amoxicillin	100 µg/mL
Cephalexin	100 µg/mL
Ciprofloxacin	100 µg/mL
Erythromycin	100 µg/mL

g. *Assay Cut-off:*

n/a

h. Specimen Stability

The specimen collection and handling instructions recommend that blood smear slides for the PlasG and PlasFV test kits should be prepared and fixed as soon as possible after venous whole blood sample collection into EDTA vacutainer tubes.

In the clinical study, blood smears were prepared from leftover EDTA venous whole blood samples that were stored at 15 – 30 °C for less than 24 hours or stored at 4 °C for less than four days. The clinical study results supported the storage of whole blood under these conditions prior to preparing blood smear slides.

To evaluate the stability of blood smear slides that have been prepared and fixed according to package insert instructions, a real-time sample stability study was conducted. Multiple slide replicates were prepared from each of four *P. falciparum* and 10 *P. vivax* positive blood samples that had different concentrations of parasites as determined by microscopic examination (low, medium, or high parasite concentrations. The slides were prepared and stored with desiccant in the dark at 15 – 30 °C. The PlasG and PlasFV test kits were used to stain selected slide replicates every three months for the first year, and once per year thereafter up to five years. All samples produced the expected test kit result at each time point without a noticeable reduction in staining intensity. The results supported the storage of fixed slides for specimens or quality controls for up to five years prior to staining.

i. Fresh versus Frozen Study

n/a

2. Comparison Studies:

a. Clinical Comparison:

See section L3

b. Matrix Equivalence Study

n/a

3. Clinical Studies:

The clinical performances of the PlasG and PlasFV test kits were evaluated in a multi-center study conducted in malaria endemic and non-endemic regions. The test kit results were compared to Giemsa thick and thin smear malaria microscopy results that followed a uniform protocol with pre-defined Giemsa adjudication among multiple readers. PCR testing followed by bi-directional sequencing was used for discrepant analysis.

Sample collection

A total of 1067 leftover venous whole blood (EDTA) samples were collected from patients with malaria-like symptoms at different times between 2013 and 2015 (See Table 9). Endemic patient ages ranged from 29 days old to 92 years old, and non-endemic patient ages ranged from two years old to 92 years old (See Tables 7 - 8). Of the 917 patient samples enrolled from malaria endemic regions, 300 sequential samples (i.e., all comers meeting inclusion criteria) were collected from a site in Mangalore India, 395 sequential samples were collected from three Kenyan sites that receive samples from western and central Kenya (one site in Kisumu, Kenya and two sites in Nairobi, Kenya), and 222 samples were collected from Iquitos, Peru. The 222 samples from Peru were selectively enrolled (non-sequential) based on Giemsa results as follows: 22 Giemsa positive samples, and an additional 100 Giemsa positive samples with 100 matched Giemsa negative samples. A total of 150 non-endemic symptomatic patient samples were collected from the United States. An additional 21 samples that were collected from Kenya were excluded from analysis as per the study protocol because the patients had received malaria treatment within the six weeks prior to sample collection.

Table 7. Patient Age Ranges – Endemic Regions

Patient Age group	Kenya	India	Peru	Total Samples Tested	Percent
29 days - < 2 years	8	2	0	10	1.1%
2 years - < 12 years	154	10	22	186	20.3%
12 years - < 18 years	41	18	12	71	7.7%
18 - < 21 years	10	20	13	43	4.7%
21 years or older	180	248	175	603	65.8%
Unknown	2	2	0	4	0.4%
Total	395	300	222	917	100%

Table 8. Patient Age Ranges – Non-Endemic Region - US

Patient Age group	Total Samples Tested	Percent
29 days - < 2 years	0	0%
2 years - < 12 years	5	3%
12 years - < 18 years	6	4%
18 - < 21 years	4	3%
21 years or older	134	89%
Unknown	1	1%
Total	150	100%

Table 9. Sample Collection Totals

Endemic/ Non-Endemic	Country	Study enrollment	# Specimens
Non-Endemic	United States	All comers	150
Endemic	India	All comers	300
	Kenya	All comers	395
	Peru	Selective	222
Total			1067

Sample testing

Giemsa thick and thin smear malaria microscopy testing was conducted at each collection site using a uniform protocol and pre-defined, expert adjudication by up to three microscopists. Venous whole blood smears prepared as per the ID-FISH protocol were evaluated by the PlasG and PlasFV test kits at three testing sites: one site in Mangalore, India (testing the Indian samples, n=300), one site in Nairobi, Kenya (testing the majority of the Kenyan samples, n=299), and the sponsor internal site in Palo Alto, CA (testing the US samples, n=150, the Peruvian samples, n=222, and the remaining Kenyan samples not previously tested, n=96). All samples were blinded and coded prior to testing, and all FISH testing and comparator method testing procedures were conducted by independent technicians. External positive and negative FISH controls were tested for each run, and no control failures were observed for the 61 days of non-consecutive clinical testing across all sites. The results are summarized below.

Non-endemic region sample results

All 150 non-endemic symptomatic patient samples from the US had negative Giemsa microscopy results, and all samples produced negative results with the PlasG and PlasFV test kits. The specificity for each test kit in this population in comparison to Giemsa microscopy was 100% (150/150) 95% CI 95.0 – 99.2%.

Malaria endemic region sample results (all comers enrollment India and Kenya)

Results from sequential (all-comers) samples from India and Kenya are presented combined (n=695, Tables 10 - 12), and also separately by collection source (India n=300, Tables 13 - 15 and Kenya n=395, Tables 16 - 18). The Kenyan sample results from different collection sites and testing sites are shown combined as the test performances were similar across sites.

PlasG FISH test kit

In comparison to expert adjudicated Giemsa microscopy for detecting any *Plasmodium sp.*, the PlasG test kit had a sensitivity point estimate greater than 95% with the lower bound of the two-sided 95% CI greater than 90% with the samples collected from India and Kenya. The PlasG test kit specificity point estimate was 92.6% for these endemic samples; however, the results of PCR/sequencing demonstrating the presence of parasite DNA in 20 of the 27 false positive samples indicate that the specificity is likely to be higher (Table 10).

PlasFV FISH test kit – P. falciparum

In comparison to expert adjudicated Giemsa microscopy for detecting and identifying *P. falciparum* with the samples collected from India and Kenya, the sensitivity of the PlasFV test kit was 97.4%(152/156) 95% CI 93.6% - 99.0%. The specificity for *P. falciparum* in this data set was 96.1%; however, PCR/sequencing results demonstrating the presence of *P. falciparum* DNA in 17 of the 20 false positive samples indicate that the specificity is likely to be higher (Table 11).

PlasFV FISH test kit – P. vivax

No *P. vivax* positive samples were collected from Kenya (Table 18). With the samples collected from India, the sensitivity of the PlasFV test kit for identifying *P. vivax* was 91.2% (156/171) 95% CI 86.0 - 94.6% in comparison to adjudicated Giemsa microscopy (Table 15). When results with prospective samples from India and Kenya are combined, the specificity of the PlasFV test kit for *P. vivax* was 98.3% (515/524) 95% CI 96.8 - 99.1%; however, PCR/sequencing results demonstrating the presence of *P. vivax* DNA in 6 of the 9 false positive samples indicate that the specificity is likely to be higher (Table 12).

Combined Sample Results – India and Kenya

Table 10. PlasG test kit vs. Giemsa microscopy - All *Plasmodium sp.*: Combined Indian and Kenyan Sample Results

		Giemsa Microscopy		
		Pos	**Neg**	**Grand Total**
PlasG FISH	**Pos**	314[b]	27[a]	341
	Neg	14	340	354
	Grand Total	328	367	695

Sens	95.7%	(314/328)	95% CI	93.0%	97.4%
Spec	92.6%	(340/367)	95% CI	89.5%	94.9%

[a] Of the 27 samples with Giemsa negative and PlasG test kit positive results, 20 samples were positive for *Plasmodium* species DNA by PCR/sequencing: 13 *P. falciparum* DNA positive, 6 *P. vivax* DNA positive, and 1 *P. malariae* DNA positive.
[b] Of the 314 concordant Giemsa positive and PlasG test kit positive results, 2 samples were positive for *P. ovale* DNA and 1 sample was positive for *P. malariae* DNA by PCR/sequencing.

Table 11. PlasFV test kit vs. Giemsa Microscopy - *P. falciparum*: Combined Indian and Kenyan Sample Results

		Giemsa Microscopy		
		***P. f.* Pos**	***P.f.* Neg**	**Grand Total**
PlasFV FISH	***P.f.* Pos**	152	21[a]	173
	***P.f.* Neg**	4	518	522
	Grand Total	156	539	695

Sens	97.4%	(152/156)	95% CI	93.6%	99.0%
Spec	96.1%	(518/539)	95% CI	94.1%	97.4%

[a] Of the 21 samples negative for *P. falciparum* by Giemsa and positive for *P. falciparum* by the PlasFV test kit, 17 samples were positive for *P. falciparum* DNA by PCR/sequencing.

Table 12. PlasFV test kit vs. Giemsa Microscopy - *P. vivax*: Combined Indian and Kenyan Sample Results

		Giemsa Microscopy		
		***P.v.* Pos**	***P.v.* Neg**	**Grand Total**
PlasFV FISH	***P.v.* Pos**	156	9[a]	165
	***P.v.* Neg**	15	515	530
	Grand Total	171	524	695

Sens	91.2%	(156/171)	95% CI	86.0%	94.6%
Spec	98.3%	(515/524)	95% CI	96.8%	99.1%

[a] Of the 9 samples negative for *P. vivax* by Giemsa negative and positive for *P. vivax* by the PlasFV test kit, 6 samples were positive for *P. vivax* DNA by PCR/sequencing. One sample that was positive for *P. falciparum* by Giemsa and positive for *P. falciparum* DNA by PCR/sequencing is included in this count of samples negative for *P. vivax* by Giemsa.

Sample Results – India

Table 13. PlasG test kit vs. Giemsa microscopy - All *Plasmodium sp.:* Indian Sample Results

		Giemsa Microscopy		
		Pos	**Neg**	**Grand Total**
PlasG FISH	**Pos**	179	9[a]	188
	Neg	11	101	112
	Grand Total	190	110	300

Sens	94.2%	(179/190)	95% CI	89.9%	96.7%
Spec	91.8%	(101/110)	95% CI	85.2%	95.6%

[a] Of the 9 samples with Giemsa negative and FISH positive results, 7 samples were positive for *Plasmodium* species DNA by PCR/sequencing: 6 samples positive for *P. vivax* DNA and 1 sample positive for *P. falciparum* DNA.

Table 14. PlasFV test kit vs. Giemsa Microscopy - *P. falciparum*: Indian Sample Results

		Giemsa Microscopy		
		***P. f.* Pos**	***P.f.* Neg**	**Grand Total**
PlasFV FISH	***P.f.* Pos**	19	5[a]	24
	***P.f.* Neg**	0	276	276
	Grand Total	19	281	300

Sens	100%	(19/19)	95% CI	83.2%	100.%
Spec	98.2%	(276/281)	95% CI	95.9%	99.2%

[a] All 5 samples with Giemsa negative and FISH positive results were positive for *P. falciparum* DNA by PCR/sequencing.

Table 15. PlasFV test kit vs. Giemsa Microscopy - *P. vivax*: Indian Sample Results

		Giemsa Microscopy		
		***P.v.* Pos**	***P.v.* Neg**	**Grand Total**
PlasFV FISH	***P.v.* Pos**	156	9[a]	165
	***P.v.* Neg**	15[b]	120	135
	Grand Total	171	129	300

Sens	91.2%	(156/171)	95% CI	86.0%	94.6%
Spec	93.0%	(120/129)	95% CI	87.3%	96.3%

[a] Of the 9 samples Giemsa negative and FISH positive for *P. vivax*, 6 samples were positive for *P. vivax* DNA by PCR/sequencing. One sample that was positive for *P. falciparum* DNA by PCR/sequencing and Giemsa positive for *P. falciparum* is included in the count of samples negative for *P. vivax* by Giemsa.

[b] Of the 15 samples that were positive for *P. vivax* by Giemsa and negative for *P. vivax* by the PlasFV test kit, 4 samples were positive for *Plasmodium sp.* by the PlasG test kit. All 4 samples were positive for *P. falciparum* DNA by PCR/sequencing; however, dual infection with *P. vivax* could not be ruled out by this method. 3 of the 4 sample were positive for *P. falciparum* by the PlasFV test kit

Sample Results – Kenya

Table 16. PlasG Kit vs. Giemsa microscopy – All *Plasmodium* sp.: Combined Kenyan Sample Results

		Giemsa Microscopy		
		Pos	**Neg**	**Grand Total**
PlasG FISH	**Pos**	135[b]	19[a]	154
	Neg	3	238	241
	Grand Total	138	257	395

Sens	97.8%	(135/138)	95% CI	93.8%	99.3%
Spec	92.6%	(238/257)	95% CI	88.7%	95.2%

[a] Of the 19 samples with Giemsa negative and positive PlasG test kit results, 13 samples were positive for *Plasmodium* species DNA by PCR/sequencing: 12 samples positive for *P. falciparum* DNA and 1 sample positive for *P. malariae* DNA.
[b] Of the 135 Giemsa positive and FISH positive results, 2 samples were positive for *P. ovale* DNA and 1 sample was positive for *P. malariae* DNA by PCR/sequencing.

Table 17. PlasFV test kit vs. Giemsa Microscopy – *P. falciparum*: Combined Kenyan Sample Results

		Giemsa Microscopy		
		***P. f.* Pos**	***P.f.* Neg**	**Grand Total**
PlasFV FISH	***P.f.* Pos**	133	16[a]	149
	***P.f.* Neg**	4[b]	242	246
	Grand Total	137	258	395

Sens	97.1%	(133/137)	95% CI	92.7%	98.9%
Spec	93.8%	(242/258)	95% CI	90.2%	96.2%

[a] Of the 16 samples with Giemsa negative and FISH positive results, 12 samples were positive for *P. falciparum* DNA by PCR/sequencing.
[b] Of the 4 samples positive for *P. falciparum* by Giemsa and negative for *P. falciparum* by the PlasFV test kit, 1 sample was positive for *Plasmodium sp.* by the PlasG test kit. This sample was positive for *P. ovale* DNA by PCR/sequencing.

Table 18. PlasFV test kit vs. Giemsa Microscopy – *P. vivax*: Combined Kenyan Sample Results

		Giemsa Microscopy		
		***P.v.* Pos**	***P.v.* Neg**	**Grand Total**
PlasFV FISH	***P.v.* Pos**	0	0	0
	***P.v.* Neg**	0	395	395
	Grand Total	0	395	395

Sens	n/a				
Spec	100%	(395/395)	95% CI	99.0%	100%

Malaria endemic region sample results (selective enrollment Peru)
The Peru sample results are presented separately as positive percent agreement and negative percent agreement (PPA, NPA) with adjudicated Giemsa microscopy because this sample set was selectively enrolled based on Giemsa results (Peru n=222, Tables 19 - 21).

PlasG FISH test kit
The PlasG test kit had a PPA of 95.9% (117/122) 95% CI 90.8 - 98.2% and a NPA of 100% (100/100) 95% CI 96.3 - 100% in comparison to Giemsa results for detecting any *Plasmodium sp.* with the samples collected from Peru (Table 19).

PlasFV FISH test kit – P. falciparum
The PlasFV test kit had a PPA of 100% (5/5) 95% CI 56.6 - 100% and a NPA of 100% (217/217) 95% CI 98.3 - 100% for *P. falciparum* in comparison to Giemsa results with the samples collected from Peru (Table 20).

PlasFV FISH test kit – P. vivax
The PlasFV test kit had a PPA of 90.6% (106/117) 95% CI 84.0 - 94.7% and a NPA of 100% (105/105) 95% CI 96.5 -100% for *P. vivax* in comparison to Giemsa results with the samples collected from Peru. Of the 11 samples reported as Giemsa positive and PlasFV test kit negative for *P. vivax,* seven samples were positive for *Plasmodium sp.* by the PlasG test kit (Table 21)

Sample Results - Peru

Table 19. PlasG test kit vs. Giemsa microscopy – All *Plasmodium sp.*: Peruvian Sample Results

		Giemsa Microscopy		
		Pos	**Neg**	**Grand Total**
PlasG FISH	**Pos**	117	0	117
	Neg	5	100	105
	Grand Total	122	100	222

PPA	95.9%	(117/122)	95% CI	90.8%	98.2%
NPA	100%	(100/100)	95% CI	96.3%	100%

Table 20. PlasFV test kit vs. Giemsa Microscopy - *P. falciparum*: Peruvian Sample Results

		Giemsa Microscopy		
		***P. f.* Pos**	***P.f.* Neg**	**Grand Total**
PlasFV FISH	***P.f.* Pos**	5	0	5
	***P.f.* Neg**	0	217	217
	Grand Total	5	217	222

PPA	100%	(5/5)	95% CI	56.6%	100%
NPA	100%	(217/217)	95% CI	98.3%	100%

Table 21. PlasFV test kit vs. Giemsa Microscopy - *P. vivax*: Peruvian Sample Results

		Giemsa Microscopy		
		***P.v.* Pos**	***P.v.* Neg**	**Grand Total**
PlasFV FISH	***P.v.* Pos**	106	0	106
	***P.v.* Neg**	11[a]	105	116
	Grand Total	117	105	222

PPA	90.6%	(106/117)	95% CI	84.0%	94.7%
NPA	100%	(105/105)	95% CI	96.5%	100%

[a] Of the 11 samples reported as Giemsa positive and FISH negative for *P. vivax* by the PlasFV test kit, 7 samples were positive for *Plasmodium sp.* by the PlasG test kit.

Positive results stratified by parasitemia
Kit performance for all positive samples combined is also presented stratified by the Giemsa microscopy quantified parasite level (PlasG FISH Tables 22 - 24, and PlasFV FISH Tables 25 - 26).

The sensitivity point estimate for the PlasG test kit for all species combined was 94.7% at parasite concentrations of 101 – 500 parasites per microliter, and 100% at concentrations above 5000 parasites per microliter (Table 22).

The sensitivity point estimate of the PlasFV test kit for *P. falciparum* was 93.3% at parasite concentrations of 101 – 500 parasites per microliter, and 100% at concentrations above 500 parasites per microliter (Table 25).

The sensitivity point estimate of the PlasFV test kit for *P. vivax* was 92.6 -97.5% at parasite concentrations between 1001 – 10,000 parasites per microliter, and 100% at higher concentrations (Table 26).

Combined Results Stratified by Parasitemia – PlasG test kit

Table 22. PlasG test kit vs. Giemsa Microscopy for All *Plasmodium* Species

Parasites/µl blood	Sensitivity	(PlasG FISH Pos/ Giemsa sp. Pos)	95% CI	
>10000	100%	(63/63)	94.3%	100%
5001-10000	100%	(99/99)	96.3%	100%
1001-5000	95.8%	(113/118)	90.5%	98.2%
501-1000	98.4%	(62/63)	91.5%	99.7%
101-500	94.7%	(71/75)	87.1%	97.9%
<100	71.9%	(23/32)	54.6%	84.4%
Total	95.8%	(431/450)	93.5%	97.3%

Table 23. Combined Results Stratified by Parasitemia. PlasG test kit vs. Giemsa Microscopy for *P. falciparum*

Parasites/µl blood	Sensitivity	(PlasG FISH Pos/ Giemsa *P.f.* Pos)	95% CI	
>10000	100%	(38/38)	90.82%	100%
5001-10000	100%	(17/17)	81.6%	100%
1001-5000	100%	(37/37)	90.6%	100%
501-1000	100%	(26/26)	87.1%	100%
101-500	96.7%	(29/30)	83.3%	99.4%
<100	84.6%	(11/13)	57.8%	95.7%
Total	98.1%	(158/161)	94.7%	99.4%

Table 24. Combined Results Stratified by Parasitemia. PlasG test kit vs. Giemsa Microscopy for *P. vivax*

Parasites/µl blood	Sensitivity	(PlasG FISH Pos/ Giemsa *P.v.* Pos)	95% CI	
>10000	100%	(25/25)	86.7%	100%
5001-10000	100%	(81/81)	95.5%	100%
1001-5000	93.8%	(76/81)	86.4%	97.3%
501-1000	97.3%	(36/37)	86.2%	99.5%
101-500	93.3%	(42/45)	82.1%	97.7%
<100	63.2%	(12/19)	41.0%	80.9%
Total	94.4%	(272/288)	91.2%	96.6%

Combined Results Stratified by Parasitemia – PlasFV test kit

Table 25. Combined Results Stratified by Parasitemia. PlasFV test kit vs. Giemsa Microscopy for *P. falciparum*

Parasites/µl blood	**Sensitivity**	**(PlasFV FISH Pos/ Giemsa *P.f.* Pos)**	**95% CI**	
>10000	100%	(38/38)	90.8%	100%
5001-10000	100%	(17/17)	81.6%	100%
1001-5000	100%	(37/37)	90.6%	100%
501-1000	100%	(26/26)	87.1%	100%
101-500	93.3%	(28/30)	78.7%	98.2%
<100	84.6%	(11/13)	57.8%	95.7%
Total	97.5%	(157/161)	93.8%	99.0%

Table 26. Combined Results Stratified by Parasitemia. PlasFV test kit vs. Giemsa Microscopy for *P. vivax*

Parasites/µl blood	**Sensitivity**	**(PlasFV FISH Pos/ Giemsa *P.v.* Pos)**	**95% CI**	
>10000	100%	(25/25)	86.7%	100%
5001-10000	97.5%	(79/81)	91.4%	99.3%
1001-5000	92.6%	(75/81)	84.8%	96.6%
501-1000	83.8%	(31/37)[b]	68.9%	92.4%
101-500	88.9%	(40/45)	76.5%	95.2%
<100	63.2%	(12/19)	41.0%	80.9%
Total	91.0%	(262/288)[a]	87.1%	93.8%

[a] Of the 26 samples overall that were positive for *P. vivax* by Giemsa and negative for *P. vivax* by the PlasFV test kit, 11 samples were positive for *Plasmodium sp.* by the PlasG test kit.
[b] Of the 6 samples in this category that were positive for *P. vivax* by Giemsa and negative for *P. vivax* by the PlasFV test kit, 2 samples were positive for *P. falciparum* DNA by PCR/sequencing.

4. Clinical cut-off**:**

   n/a. This is a qualitative test.

5. Expected values/Reference range:

   n/a

M. Instrument Name

n/a

N. System Descriptions:

1. Modes of Operation:
   n/a

2. Software:

   n/a

3. Specimen Identification:

   n/a

4. Specimen Sampling and Handling:

   n/a

5. Calibration:

   n/a

6. Quality Control:

   See section L1c.

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

Fluorescence Microscope Illumination Comparison

A study was conducted to evaluate whether blood smears stained with PlasG and PlasFV test kit reagents can provide equivalent results when visualized with fluorescence microscopes that use either mercury arc lamp or LED fluorescence illumination. A sample panel consisting of high and low concentration *P. falciparum*, high and low concentration *P. vivax*, and negative blood samples was prepared as blood smear slides, and the smears were stained with PlasG and PlasFV test kit reagents. The slides were observed on a microscope with LED

illumination and on a microscope with mercury arc lamp illumination. No false positive results were observed. All parasite positive slides produced the expected results with both illumination sources, and no signal bleed-through was observed for either illumination source. All other analytical and clinical samples were conducted with LED illumination. The results support the use of either LED or mercury arc lamp illumination with the PlasG and PlasFV test kits.

P. Proposed Labeling:

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

1. Indications for Use statement (Special Control 5):

The Indications for Use statement in the device labeling must include a statement that the device is intended to be used in conjunction with clinical history, signs, symptoms, and the results of other diagnostic testing.

2. Interpretation of results and acceptance criteria (Special Control 6)

A detailed explanation of the interpretation of results and acceptance criteria for any quality control testing must be included in the device's 21 CFR 809.10(b)(9) compliant labeling.

3. Negative results limitation (Special Control 7)

The device labeling must include a limitation that negative results do not preclude the possibility of infection.

Q. Identified Risks and Required Mitigations:

Identified Risks to Health	Identified Mitigations (See Section S below for Special Controls)
Incorrect identification or lack of identification of a pathogenic microorganism by the device can lead to improper patient management	General Controls and Special Controls (1), (2), (3), (4), (5), (6), and (7)
Failure to correctly interpret test results	General Controls and Special Controls (5), (6), and (7)
Failure to correctly operate the instrument	General Controls and Special Controls (5) and (6)

R. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	• The PlasG and PlasFV FISH test kits are the first assays to use fluorescent in situ hybridization to detect and identify malaria nucleic acids directly in blood smears prepared from venous EDTA blood samples. • The PlasG and PlasFV FISH test kits can assist microscopists with detecting and identifying malaria parasites, and may be particularly valuable at sites where personnel have less experience or training in interpreting malaria smears. • The PlasG and PlasFV FISH test kits assist in distinguishing between *Plasmodium falciparum* and *Plasmodium vivax* infections. • In comparison to expert adjudicated Giemsa microscopy, the PlasG and PlasFV FISH test kits demonstrated sensitivity greater than 95% for the identification of *Plasmodium spp.* and *P. falciparum* infections, and sensitivity greater than 90% for the identification of *P. vivax* infections.
Summary of the Risk(s)	• False positive results and false negative results are the primary risks associated with use of the PlasG and PlasFV FISH test kits. • A false positive result may lead to unnecessary antimalarial therapy, with associated adverse events. • A false negative result may result in a delay of antimalarial therapy, with subsequent worsening of infection. A false negative result may also result a missed opportunity to provide antimalarial therapy to eliminate malaria hypnozoites, the dormant liver form of *P. vivax*, with subsequent relapse of infection.
Summary of Other Factors	None.

Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of the PlasG and PlasFV FISH test kits outweigh the potential risks in light of the listed special controls and applicable general controls, including design controls. The PlasG and PlasFV FISH test kits are the first assays to use fluorescent in situ hybridization to detect and identify malaria nucleic acids in blood smear samples, and are likely to benefit patients by assisting with the diagnosis and identification of malaria infections. The high performance observed during the pivotal clinical trials in comparison to adjudicated Giemsa microscopy, and the proposed special controls suggest that errors will be uncommon and will be well mitigated by current laboratory practices, including traditional microscopy and product labelling. The PlasG and PlasFV FISH test kits could provide substantial benefits as an aid in the diagnosis of malaria infections.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3367. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PYN

Device Type: Device to detect and identify microbial nucleic acids by FISH in clinical specimens

Class: II (special controls)

Regulation: 21 CFR 866.3367

(a) Identification. A device to detect and identify microbial nucleic acids by fluorescence in situ hybridization (FISH) in clinical specimens is an in vitro diagnostic device intended for the detection and identification of microbial pathogens in specimens collected from patients with signs and symptoms of infection. The device is intended to aid in the diagnosis of human disease in conjunction with clinical signs and symptoms and other laboratory findings.

(b) Classification. Class II (special controls). A device to detect and identify microbial nucleic acids by FISH in clinical specimens must comply with the following special controls:

1) Premarket notification submissions must include detailed device description documentation, including the device components, instrument requirements, ancillary reagents required but not provided, and a detailed explanation of the methodology including all pre-analytical methods for processing of specimens, probe sequences, and rationale for probe sequence selection.
2) Premarket notification submissions must include a detailed description of the fluorophores, signal source, detection mechanism and method of result interpretation.
3) Premarket notification submissions must include detailed documentation from the following analytical studies: analytical sensitivity (Limit of Detection),

inclusivity, reproducibility, interference, cross reactivity, and specimen stability.

4) Premarket notification submissions must include detailed documentation from a clinical study that includes prospective (sequential) samples. The study, performed on a study population consistent with the intended use population, must compare the device performance to results obtained from appropriate and well-accepted comparator methods.
5) The 21 CFR 809.10(b)(2) compliant labeling must include a statement that the device is intended to be used in conjunction with clinical history, signs, symptoms, and the results of other diagnostic testing.
6) The 21 CFR 809.10(b)(9) compliant labeling must include a detailed explanation of the interpretation of results and acceptance criteria for any quality control testing.
7) The 21 CFR 809.10(b)(5)(ii) compliant labeling must include a limitation that negative results do not preclude the possibility of infection.