510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k132066

B. Purpose for Submission:

New device

C. Measurand:

Matrix metalloproteinase-9 (MMP-9) in human tears

D. Type of Test:

Qualitative immunoassay

E. Applicant:

Rapid Pathogen Screening, Inc.

F. Proprietary and Established Names:

InflammaDry
InflammaDry External Controls

G. Regulatory Information:

1. Regulation section:

   862.1540 Osmolality Test System
   862.1660 Quality control material (assayed and unassayed)

2. Classification:

   Class I, exempt; subject to limitations of exemption 21 CFR § 862.9 (b) and (c)(9)
   Class I, reserved

3. Product code:

   PFQ, JJX

4. Panel:

   75, Chemistry

H. Intended Use:

1. Intended use(s):

   See indications for use below

2. Indication(s) for use:

   InflammaDry is a rapid, immunoassay test for the visual, qualitative *in vitro* detection of elevated levels of the MMP-9 protein in human tears from patients suspected of having dry eye to aid in the diagnosis of dry eye in conjunction with other methods of clinical evaluation. This test is intended for prescription use at point-of-care sites.

   InflammaDry External Controls are QC materials used for verifying the performance of the InflammaDry test reagents and assay. These controls can also be used to assist in operator training and troubleshooting invalid results.

3. Special conditions for use statement(s):

   - For prescription use only
   - For point-of-care use
   - Slit-lamp biomicroscopy is required to eliminate patients with active intraocular inflammation.
   - InflammaDry requires a visual readout. Do not interpret the test result if you have color-impaired vision.
   - The Dacron material used in the Sampling Fleece may cause allergic reactions for some people.
   - InflammaDry should be performed prior to instilling ocular anesthetic, topical dyes, or performing Schrimer testing.
   - Certain medications may cause erroneous results if used immediately before taking a sample. If ocular anesthetic or any other topical medication has been applied to the eye, wait at least 2 hrs prior to collecting a sample.

4. Special instrument requirements:

   Not applicable; this is a visually read single use device

I. Device Description:

InflammaDry™ consists of three (3) parts: a sterile sample collector, an immunoassay test strip in a plastic test cassette housing, and buffer in a vial. The separately packaged and sterile sample collector has a contoured end with a dacron fleece to collect the tear sample from the inside of the lower eyelid. The plastic housing of the test cassette body protects the strip from unintended physical influence. Additionally, the housing guarantees correct sample transfer onto the lateral flow assay strip. The buffer vial contains a buffered salt solution containing: 200 mM Tris, 10% Fish 81 (sea block), 0.8% Tergitol, 100 mM NaCl, 0.1% Sodium Azide, 0.0126% Gentamycin and pH 9.5 ± 0.05. The buffer functions as the solution that initiates the test, carries antigen through a microfiltration process to remove unwanted cellular debris, and transports the immune complex and the control conjugate to the "Test" and "Control" lines on the test strip membrane.

The InflammaDry external controls are supplies as lyophilized powder in small glass vials with screw caps. A soft and pliable plastic dropper bottle filled with DI water diluent is provided with each set of external controls to reconstitute the lyophilized control materials.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Occusense Inc. TearLab Osmolarity System and TearLab Control Solutions

2. Predicate 510(k) number(s):

   k083184

3. Comparison with predicate:

Similarities/Difference		
Item	Candidate Device	Predicate Device
Indications for Use	Measurement of tear fluid from patients suspected of having dry eye to aid in the diagnosis of dry eye in conjunction with other methods of clinical evaluation.	Same
Materials	Plastic housings, membrane, glass fiber absorbent tip and waste pad, foil pouches, MMP-9 antibody gold conjugate, dacron fleece and buffer solution. Phenol red dye added to	The device consists of the following components and accessories: One TearLab Reader, Two TearLab Pens, Two TearLab

Similarities/Difference		
Item	Candidate Device	Predicate Device
	the sample collector dacron fleece.	Electronic Check Cards, Single Use TearLab Osmolarity Test cards and TearLab Control Solutions.
Sterile	Yes, sample collector by gamma radiation	No; test cards are hygienically clean
Kit composition	Sample collector and test cassette sealed in foil pouches and a vial of buffer	The device consists of the following components and accessories: One TearLab Reader, Two TearLab Pens, Two TearLab Electronic Check Cards, Single Use TearLab Osmolarity Test cards and TearLab Control Solutions.
Technology	Lateral Flow Immunoassay	The TearLab Osmolarity Test utilizes an impedance measurement of tear fluid to provide a calculated measurement of osmolarity
Form	Lyophilized	Liquid
Intended use of the controls	Verify performance of InflammaDry test reagent and assay; assist with operator training; troubleshoot invalid results	Monitor day-to-day test variation; lot-to-lot kit performance; assist with operator training; troubleshoot invalid results
Test Results	Qualitative	Quantitative

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

11137-1; ANSI/AAMI/ISO Sterilization of health care products – Radiation -Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices, 2006/(R) 2010

11137-2; ANSI/AAMI/ISO Sterilization of health care products – Radiation – Part 2: Establishing the Sterilization Dose 2012

14971; ISO Medical Devices – Application of Risk Management to Medical Devices

L. Test Principle:

The InflammaDry test is based on the principle of lateral flow immunoassay using direct sampling micro-filtration technology. Matrix Metalloproteinase -9 (MMP-9) present in the tear fluid is captured between two highly specific anti-MMP-9 antibodies: a monoclonal

mouse anti-MMP-9 antibody and a polyclonal goat antihuman antibody. This antigen-antibody complex is captured by NeutrAvidin immobilized as the test line. The formation of a blue color line at the control zone line with a red color line at the test zone line is considered as a positive result, a blue color line at the control zone only is considered as a negative result, if a blue color line in the control zone does not appear the test is considered invalid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were performed at three point-of-care sites using stabilizing buffer spiked to the following concentrations: negative (zero), cutoff, +/-25%, and +/-50%, of the cutoff. The samples were aliquoted, randomized and blinded, then given to each site. A total of 120 determinations were made at each concentration. Testing was performed in duplicate twice a day over 5 days by 6 intended use operators (2/site). The intended users performed the testing by following the instructions for use. Sample concentrations were confirmed by LC/MS/MS.

		Negative	-50%	-25%	Cutoff	+25%	+50%
		Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos	Neg/Pos
Site 1	Op 1	20/0	19/1	18/2	4/16	0/20	0/20
	Op 2	20/0	19/1	10/10	4/16	2/18	0/20
Site 2	Op 1	20/0	20/0	17/3	10/10	3/17	0/20
	Op 2	20/0	20/0	18/2	12/8	2/18	3/17
Site 3	Op 1	20/0	20/0	19/1	10/10	2/18	0/20
	Op 2	20/0	20/0	18/2	12/8	2/18	0/20
Combined		120/0	118/2	100/20	52/68	11/109	3/117

b. Linearity/assay reportable range:

Not applicable. The assay is intended for qualitative use.

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

InflammaDry has built-in procedural controls. The appearance of the control line indicates the correct application of sample volume

InflammaDry External Controls consists of 2 levels (a positive and a negative) of QC materials: Positive controls are prepared by spiking MMP-9 in stabilizing buffer solution with known concentration of MMP-9 antigen which are traceable to a MMP-

9 monomer form of progelatinase B from human blood. The positive control concentrations are confirmed by LC/MS/MS. Negative control is recombinant MMP-9 in stabilizing buffer solution containing no drug.

External controls solutions are not supplied with this device but can be purchased separately from Rapid Screening Diagnostic Inc. Users are advised to follow federal, state and local guidelines for QC testing requirements.

Stability:

Accelerated and Real time studies have been conducted for the control material. Protocols and acceptance criteria were described and found to be acceptable. The manufacturer claims that when stored un-opened at room temperature, the product is stable until expiration date which is 24 months. Open vial stability the controls should be used on the same day they are reconstituted.

d. Detection limit:

Not applicable, this is a qualitative assay.

e. Analytical specificity:

Cross-reactivity of the test with various microorganisms and cytokines was assessed by adding 15 µl of potential cross-reactant to a sample collector fleece then running the test as per the directions. Microorganism concentrations ranged from 500,000 and 1,500,000 microorganisms (virus, bacteria) per ml of supernatant and cytokines were tested at 150 ng/mL. All isolates were cultured from human specimen. There were no positive tests observed for the following:

Microorganism (virus, bacteria) and ocular enzymes:
Adenovirus, IgE, Staphylococcus aureus, Methicillin resistant Staphylococcus aureus (MRSA), Moraxella catarrhalis, Haemophilus influenza, Staphylococcus epidermis, Streptococcus pneumonia and Pseudomonas aeroginosa.

Cytokines:
Matrix metalloproteinase: 1, 2 and 3
Tissue Inhibitor of MMP: MMP-1, MMP-2

Interference study:
The effect of different eye drops on the analytical sensitivity and specificity of the InflammaDry ™ tests was determined. The eye drops were evaluated with two different MMP-9 antigen concentrations, C_5 (C_{50} – 25%) and C_{95} (C_{50} + 25%) in duplicate. Influence of the relevant proteins on sensitivity and specificity is also examined. Any positive or negative interfering substance identified by this testing was re-tested using C_{50} – 50%, and C_{50} +50%.

The following eye medications were tested for interference in human tears at the cutoff level and the respective medication. The following medications did not show any interferences:

Alcaine Alcon, "Azopt"-Alcon, Econopred Alcon, "Nevanac"-Alcon, "Pataday"-Alcon, Systaine"-Alcon, Tobra Dex, "Travatan"-Alcon, Alcon, Vigamox Alcon, "Acular LS"-Allergan, Alphagan Allergan, "Combigan"-Allergan, Elastat Allergan, "FML"-Allergan Lastacaft –Allergan, Lumigan Allergan, Optive"-Allergan, Pred Forte"-Allergan, Refresh Liquigel, Refreash Tears Allergan, Zymar Allergan, "Blink Tears"-Amo, Thera Tears AVS, Alrex B&L, Lotemax B&L, "Zylet"-Bausch&Lomb, Gentamycin Sulfate-Falcon, Polymyxin B sulfate Falcon, "Timolol"-Falcon, "AzaSite"-Inspire, Bepreve-Ista, "Xibrom"-Ista, Optivar MedPDEnte, Truspot Merck, GenTeal"-Novartis, "Voltaren"-Novartis, "Zaditor"-Novartis, Visine Pfizer, Xalatan Pharmacia, human IgA (1 mg/ml), Sigma-Aldrich, human lactoferrin (1 mg/ml), Sigma-Aldrich, Transferrin (1 mg/ml), Betimol Vistakon

However, the following medications show false positive or false negative results: Vistakon, Iquix; Vistakon, Quixin; Wilson, Proparacaine; and Trusopt.

Therefore, sponsor has the following limitations in the labeling:

Patients should not be tested with InflammaDry if the following medications were administrated into the eyes within 2 hours of the testing of the ImflammaDry. Interferences medications: Vistakon, Iquix; Vistakon, Quixin; Wilson, Proparacaine; and Trusopt.

Certain medications such as systemic immunodulators, topical or oral steroids, cyclosporine, tetracycline and topical azithromycin are known to inhibit metalloproteinase activity. Use of these medications may lead to false negative results.

f. Assay cut-off:

The cutoff study performed used increments from 5 ng/mL to 70 ng/mL. The study was conducted with 10 operators testing 10 replicates using stabilizing buffer sample. The results are presented in the table below:

Concentration (ng/mL)	Negative	Positive
5	100	0
10 (-75%)	93	7
20 (-50%)	97	3
30 (-25%)	83	17
35	65	35
40 (cutoff)	48	52
50 (25%)	5	95
60 (50%)	0	100
70 (75%)	0	100

2. Comparison studies:

a. Method comparison with predicate device:

See clinical study in 3.c. below.

b. Matrix comparison:

Not applicable. The assay is intended for only one sample matrix.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

The InflammaDry Test underwent a clinical evaluation to determine the negative and positive agreement of the InflammaDry with clinical assessment of dry eye. The study design was a prospective, sequential, masked, clinical trial. Those patients who were clinically determined by an ophthalmic clinician to meet enrollment criteria were included in the study. The study enrolled 237 patients consisting of 164 females and 73 males between the ages of 18 and 94 years old with an average age of 53 years. Patients presented from both private practices and academic centers from various regions across the country. Although 257 patients were recruited, seventeen (17) patients were excluded for a protocol deviation.

The protocol deviation involved patients receiving topical ocular anesthetic prior to the evaluation of the tear break up time (TBUT) and corneal staining, potentially accelerating the TBUT and inducing corneal staining.

Study testing was done on the subject's more symptomatic eye (if no difference existed symptomatically between the two eyes, the right eye was tested). Each subject underwent the following sequence of testing: Each patient had the following tests performed: InflammaDry, tear break up time (TBUT), Schirmer tear testing, and corneal staining.

The InflammaDry was compared to the clinical assessment in the table below. Derived from the DEWS criteria, the clinical assessment was developed to represent a combination of symptoms and signs. The pivotal clinical trial used the same metrics for TBUT, Schirmer tear testing, and

corneal staining as described in the DEWS criteria, however, conjunctival injection, conjunctival staining, and the presence of meibomian disease were not tested or used to characterize the severity of dry eye disease. In general, the worst severity for any sign tested determined the overall severity. Symptoms are known to be poorly correlated with signs with even the most severe dry eye patients often reporting little to no symptoms. Patients were categorized to the highest severity level at which all required criteria are satisfied. Patients who do not meet all the required clinical criteria for a given severity grade will be considered to be at the next lower grade.

Clinical Testing	Negative Control	Mild Grade 1	Moderate Grade 2	Moderately Severe Grade 3	Sever Grade 4
OSDI score	≤ 13	≥13	≥13	≥13	≥13
TBUT (sec)	>10	<10	<10	≤5	0 (immediate)
Schirmer (mm/5 min)	>10	<10	<10	≤5	≤2
Staining (0-5)	None	None	1-2	3	≥4

Clinical Results

Site 1	Grade					Total
	4	3	2	1	0	
InflammaDry +	0	0	15	41	1	57
InflammaDry -	0	0	0	2	31	33
Total	0	0	15	43	32	90

Site 2	Grade					Total
	4	3	2	1	0	
InflammaDry +	0	7	21	1	1	30
InflammaDry -	0	0	0	7	47	55
Total	0	7	21	9	48	85

Site 3	Grade					Total
	4	3	2	1	0	
InflammaDry +	0	0	4	4	0	8
InflammaDry -	0	0	3	1	0	4
Total	0	0	7	5	0	12

Site 4	Grade					Total
	4	3	2	1	0	
InflammaDry +	1	11	20	1	0	33
InflammaDry -	0	2	13	2	0	17
Total	1	13	33	3	0	50

Grade is assessed based on OSDI, TBUT, Schirmer tear testing, and corneal staining as described

in the DEWS criteria, however, conjunctival injection, conjunctival staining, and the presence of meibomian disease were not tested or used to characterize the severity of dry eye disease.

- Grade 0 (Negative Control) is when OSDSI is ≤13, TBUT is >10 seconds, Schirmer is >10mm, Staining is none.
- Grade 1 (Mild) is when OSDI score is ≥ 13, TBUT is <10 seconds, Schirmer test is <10 mm, Staining is none.
- Grade 2 (Moderate) is when OSDSI is ≥13, TBUT is ≤10 seconds, Schirmer is ≤10 mm, Staining is 1-2.
- Grade 3 (Moderately Severe) is when OSDSI is ≥13, TBUT is <5 seconds, Schirmer is ≤5 mm, Staining is 3.
- Grade 4 (Severe) is when OSDSI is ≥13, TBUT is 0 seconds (immediate), Schirmer is ≤2 mm, Staining is ≥4.

Device Performance:
The multicenter clinical study depicted below demonstrated the following range of performance: Positive Agreement 66%-97% and Negative Agreement 97%-98%. At 2 sites Negative Agreement could not be calculated because there were no subjects without dry eye.

N=237			Clinical Assessment ODSI** + TBUT + Schirmer + Staining		Positive % agreement 95% confidence interval	Negative % agreement 95% confidence interval
			Positive	Negative		
Site 1	InflammaDry	Positive	56	1	97% (56/58) (88%, 99%)	97% (31/32) (84%, 99%)
		Negative	2	31		
Site 2	InflammaDry	Positive	29	1	76% (29/37) (62%, 90%)	98% (47/48) (89%, 100%)
		Negative	8	47		
Site 3	InflammaDry	Positive	8	0	67% (8/12) (39%, 86%)	N/A*
		Negative	4	0		
Site 4	InflammaDry	Positive	33	0	66% (33/50) (51%, 79%)	N/A*
		Negative	17	0		

*N/A = not available. Negative Agreement cannot be calculated because there were no subjects without dry eye.

** *11 patients were assessed to be positive for mild dry eye based on the OSDI (OSDI ≥ 13) without any associated positive objective test results*

4. Clinical cut-off:

   40 ng/mL

5. Expected values/Reference range:

Based on the literature the sponsor claims that the normal levels of MMP-9 in human tears range from 3 ng/mL to 40 ng/mL.

Literature:

1. Acera A, Rocha G, Vecino E, et al. Inflammatory markers in the tears of patients with ocular surface disease. Ophthalmic Res. 2008 Oct; 40(6):315-21.

2. Chotikavanich S, de Paiva CS, Li de Q, et al. Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. Invest Ophthalmol Vis Sci. 2009 Jul; 50(7):3203-9.

3. Solomon A, Dursun D, Liu Z, et al. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci. 2001;42(10):2283-92.

4. Leonardi A, Brun P, Abatangelo G, et al. Tear levels and activity of matrix metalloproteinase (MMP)-1 and MMP-9 in vernal keratoconjunctivitis. Invest Ophthalmol Vis Sci. 2003;44(7):3052-8.

5. Lema I, Sobrino T, Durán JA, et al. Subclinical keratoconus and inflammatory molecules from tears. Br J Ophthalmol. 2009;93(6):820-4.

6. Honda N, Miyai T, Nejima R, et al. Effect of latanoprost on the expression of matrix metalloproteinases and tissue inhibitor of metalloproteinase 1 on the ocular surface. Arch Ophthalmol. 2010;128(4):466-71.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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