Development of a Multiplexed Immunochromatographic Assay for Detection of Acute Bacterial Sinusitis

Oriana Hawkins[1], Oscar Okiya[1], Jack Shirajian[2], Joseph Skraba[1]

[1]Entvantage Diagnostics, Austin, TX, [2]Kestrel Biosciences, Carlsbad, CA

Abstract

Background: Annually, 29 million people are diagnosed with sinusitis in the U.S. and 80% receive a prescription for antibiotics. Although less than 10% of sinusitis cases are caused by bacteria, sinusitis accounts for more adult antibiotic prescriptions than any other outpatient diagnosis. Currently, there are no products available to diagnose bacterial sinusitis at the Point of Care (POC). With the looming threat of antibiotic resistance and 1 in 1000 patients suffering serious, sometimes life-threatening adverse effects due to antibiotics, physicians need better tools to diagnose and inform the clinical management of patients with sinusitis. Entvantage Diagnostics is developing the first multiplexed, Point of Care (POC) test for rapid diagnosis of bacterial sinusitis in the primary care and urgent care setting where 90% of sinusitis patients are seen. **Methods:** Mouse monoclonal antibodies were generated or licensed for each of three pathogens responsible for >90% of bacterial sinusitis; non-typeable *H. influenzae* (NTHI), *M. catarrhalis*, and *S. pneumoniae*. Antibody pairs were initially selected for the final assay prototype based on inclusivity, analytical sensitivity, analytical specificity, and interference by enzyme-linked immunosorbent assay (ELISA). Prior to licensing from the Respiratory Diseases Branch of the CDC, Anti-S. pneumoniae antibodies had screened positive for recognition of 90 serotypes and demonstrated minimal crossreactivity with 22 genera and 29 species of respiratory pathogens and commensals. Inclusivity: Bacterial lysates were generated using a hyperosmotic lysis buffer containing an anionic detergent, a chaotrope, and protease inhibitor and utilized as antigen by ELISA. Anti-*H. influenzae* OMP-P5 specific antibodies were selected for positive recognition of two reference strains, 5 clinical isolates, and an OMP-P5 knockout. Anti-*M. catarrhalis* CD antibodies were selected for recognition of a reference strain. Analytical Sensitivity: The target limit of detection for the final assay was $1x10^4$ CFU/ml. Antigen was diluted from $1x10^7$- $1x10^2$ CFU/ml and interrogated with the antibody pairs. Antibody pairs that met this specification were selected for additional testing. Analytical Specificity: Down-selected antibody pairs were further screened for reactivity to lysates of 12 bacterial species commonly found in the nasal cavity, including *S. aureus* and *S. epidermidis*. Those demonstrating no crossreactivity were selected for prototyping of individual lateral flow-based immunochromatographic assays. **Results:** These prototyped assays were tested in an IRB approved pilot study composed of 7 symptomatic patients, 15 healthy participants, and 22 contrived samples. Bacterial culture of endoscopically guided swab of the Middle Meatus served as the comparator method. Specificity for *H. influenzae, M. catarrhalis* and *S. pneumoniae* were 94 %, 86% and 93%, respectively. Sensitivity for *M. catarrhalis H. influenzae,* and *S. pneumoniae* were 87.5%, Not determined due to no true positives, and 100%, respectively. **Conclusion:** The results of this study support full validation and clinical assessment of this product. This rapid diagnostic will improve patient care by equipping health care providers with a means to quickly and accurately diagnose patients with sinusitis who otherwise would be treated empirically. We acknowledge the contribution of the Respiratory Diseases Branch, Division of Bacterial Diseases, CDC.

Introduction

Sinusitis is a common illness with a heavy economic burden estimated at >$8 billion, including 70 million lost or restricted work days. Nearly 29 million patients in the U.S. and 43 million in Europe receive a diagnosis of sinusitis each year. Acute Rhinosinusitis (ARS) is the inflammation of the mucosal lining of the paranasal sinuses and the nasal passage lasting fewer than 4 weeks (Fig.1). Symptoms may include fever, facial pain, purulent nasal discharge and fatigue. ARS may be caused by allergens, irritants, or infection by viruses, bacteria or fungi. The most common etiology of ARS is viral, with only 2-10% of cases caused by bacteria. Acute Bacterial Sinusitis (ABRS) can develop secondary to a viral infection, when inflammation obstructs the sinus ostium and impedes clearance of mucus. While only a small percentage of sinusitis is bacterial, it accounts for more adult antibiotic prescriptions than any other outpatient diagnosis. Physicians prescribe antibiotics for >80% of visits due to ARS and in 70% of chronic sinusitis cases.

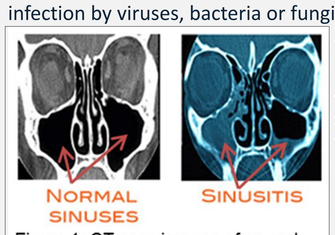


Figure 1: CT scan images of normal sinuses and those with sinusitis

It is clear that antibiotics are overprescribed for sinusitis, where they are ineffective and can cause patients significant harm. Antibiotics cause the largest number of medication-related adverse events and 20% of emergency room visits for adverse drug reactions (ADR). In addition, overuse of antibiotics for acute respiratory tract infections is a major contributor to antibiotic resistance. Despite the danger, patients often visit healthcare clinics with a predisposition to receive antibiotics without fully appreciating the need or risks. In a market survey , Primary Care Physicians (PCP) and Otolaryngologists (ENT) indicated that their greatest concern was the overuse of antibiotics, but tension between the provider and patient led 70% to prescribe antibiotics for almost every sinusitis case. Rapid diagnostic tools are effective at reducing the number of antibiotics prescribed for common ailments. Often healthcare providers lack point-of-care (POC) diagnostic tools and are forced to rely on imprecise methods to make diagnostic decisions or refer patients for complex procedures under a specialist's care.

Entvantage Dx has developed the first rapid, point-of-care diagnostic test kit to identify the three pathogens responsible for bacterial sinusitis. Included in this test kit (Fig. 2), is a specialized mucus collection device that allows for sampling of the middle meatus, the drainage site of the sinuses, without the need for an endoscope or specialized training. The sample collection device and assay were evaluated in a pilot clinical study to assess patient comfort during sampling and sensitivity of the assay compared to culture.

SinuTest Assay

Figure 2: SinuTest Assay Kit.
2A) The Mucus Collection Device allows the user to easily collect mucus from the Middle Meatus (2B) without the need for an endoscope. The critical dimensions, depth and angle of insertion of the collection device were determined by a 78 patient CT study and validated in 3 cadaver studies and a pilot clinical study. 2C) Following a brief lysis and dilution step, the sample is applied the Bacterial Assay test cassette. The SinuTest has a processing and time to read of 10-12 minutes.

Figure 2A: Mucus Collection Device

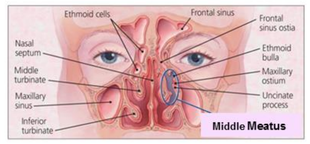


Figure 2B: Human Sinus Complex

Figure 2C: Bacterial Assay

Results

Figure 3: Inclusivity screen for *H. influenzae OMP-P5* monoclonal antibody (mAb) candidates. ELISA plates were coated with bacterial lysates from 4 clinical isolates, a reference and an OMP-P5 knockout strain. Results for 6 mAbs are shown. Reactive mAbs were selected for subsequent screens.

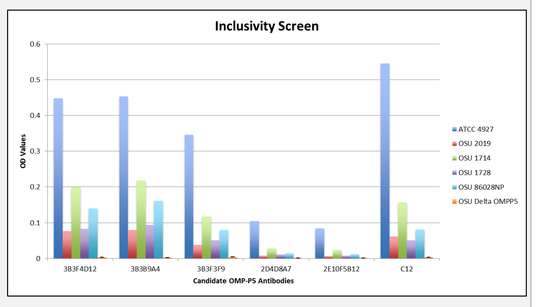


Figure 4: Analytical Specificity. Down-selected antibody pairs were screened against 12 common bacterial species (7 shown) found in the nasal cavity. ELISA plates were coated with bacterial lysates. mAbs with specific reactivity were selected for prototyping.

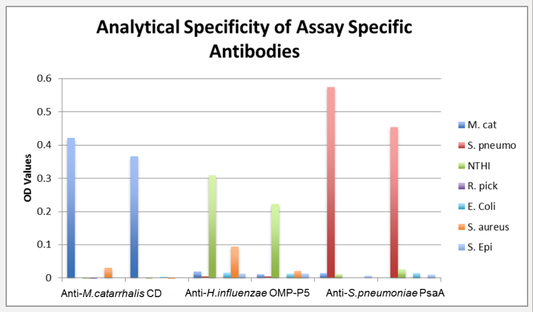
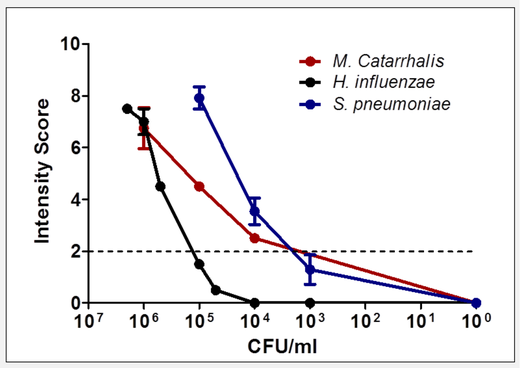


Figure 5: Analytical performance of the three individual assays. Individual assay strips were prototyped and tested for target reactivity and analytical sensitivity using lysates, native and contrived specimens, prior to multiplexing. Specimens were collected on flocked swabs, lysed and diluted 1:5 in proprietary lysis and dilution buffers. 70µl of sample was applied to each strip.



Assay Interference: Sensitivity of the assays to the presence of 12 over-the -counter and prescription intra-nasal or oral drugs in the sample was determined by ELISA and qualitative assessment using the prototype assay strips (Table 1). Crest Pro-health mouthwash significantly inhibited all 3 assays. Prescription antibiotics, mupirocin and tobramycin also inhibited the assays. It is unlikely that these agents will pose a substantial impediment to adoption of the assay kit, due to a low likelihood of encountering the agents in the middle meatus or the target patient population.

Table 1: Sensitivity of the assays to possible interfering substances.

Possible Interfering Substances	H. influenzae			M. catarrhalis			S. pneumoniae		
Concentration in Sample	10%	1%	0.10%	10%	1%	0.10%	10%	1%	0.10%
Listerine Mouthwash	-	-	-	-	-	-	-	-	-
Biotene Mouth Wash	-	-	-	-	-	-	-	-	-
Crest Pro-Health Mouthwash	+	-	-	+	+	+	+	+	-
Flonase – Fluticasone propionate	+	-	-	-	-	-	-	-	-
Simply Saline Nasal Mist	-	-	-	-	-	-	-	-	-
Afrin - Oxymetazoline	+	-	-	-	-	-	-	-	-
4 Way Nasal Spray -Phenylephrine	+	-	-	-	-	-	-	-	-
Albuterol Sulfate solution 0.083%	-	-	-	-	-	-	-	-	-
Flunisolide 0.025%	-	-	-	-	-	-	-	-	-
Mupirocin 2% ointment	+	-	-	+	-	-	-	-	-
Tobramycin solution 40 mg/ml	+	+	+	+	-	-	-	-	-
Nasacort – Triamcinolone Acetonide	-	-	-	-	-	-	-	-	-

+ = positive interference; - = no significant interference

Results

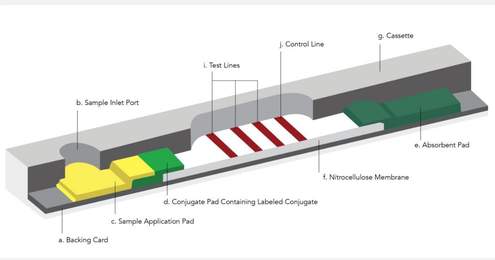


Figure 6: Diagram of the multiplexed test strip

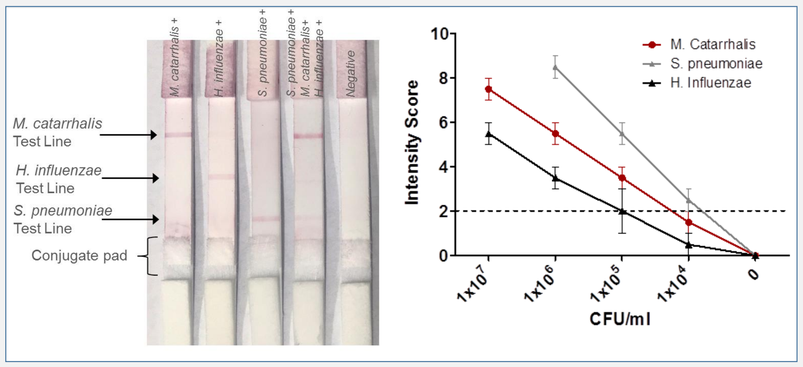


Figure 7: Prototype Multiplex assay test strips. A) Strips were tested for target reactivity and analytical sensitivity using lysates, native and contrived specimens. Specimens were collected on flocked swabs, lysed and diluted 1:5 in proprietary lysis and dilution buffers. 70µl of sample was applied to each strip. B) Analytical sensitivity for each assay falls between $1x10^4$- $5x10^5$ CFU/ml of sample.

Pilot Clinical Trial: Prototyped assays were tested in an IRB approved pilot study including 7 symptomatic patients, 15 healthy participants, and 22 contrived samples. The goals of the trial were to assess patient comfort during specimen collection and to compare the in-office test kit to bacterial culture of an endoscopically guided swab of the Middle Meatus. Information on the study participants and the relative discomfort is provide in Table 2. Table 3 provides analytical performance of the assay kit vs. culture.

Table 2: Pilot-Trial participant information

	Participants	%
n	22	
Antibiotics <90 days	0	0
Previous sinus surgery	2	9
Sinonasal symptoms ≥10d	7	32
Discomfort <4 FACES	18	82
Willing to repeat	18	82

Table 3: Pilot-Trial and contrived specimen study

		Specificity	Sensitivity
H. influenzae assay +	1/22	94%	ND
H. influenzae False +	1/22		
H. influenzae False -	0/22		
M. catarrhalis assay +	8/22	86%	87.5%
M. catarrhalis False +	1/22		
M. catarrhalis False -	4/22		
S. pneumoniae assay +	4/44	93%	100%
S. pneumoniae False +	3/44		
S. pneumoniae False -	0/44		

specificity = (100% x TN/(FP+TN)
sensitivity =(100% x TP/(TP+FN)
ND = Not Done due to lack of True Positives

Conclusions

- The multiplexed SinuTest assay is specific to the target antigens.
- SinuTest detects the bacteria at clinically relevant levels.
- Preliminary clinical results demonstrate good concordance with endoscopic middle meatal swab culture, supporting a full clinical evaluation for 510(k) and CLIA waiver submissions.
- Specimen collection caused no more discomfort than an endoscopically guided swab, with >80% of participants willing to repeat the procedure.