Dermal Microbiome-Immunology Assay

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Tradename: AC Dermal Respiratory Factor Advanced PF

Code: 20219PF

CAS #: 7732-18-5 & 8013-01-2

Test Request Form #: 9692

Lot #: N220406A

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
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Test Performed:
Dermal Microbiome-Immunology Assay

Introduction

Dendritic cells (DCs) are a small group of heterogeneous cells residing in the epidermis and dermis known as the gatekeepers of the immune system. As antigen-presenting cells, DCs recognize foreign pathogens and initiate an immune response. In response to environmental signals, DCs are activated and communicate with the rest of the immune system through paracrine signaling by releasing specific molecules which alters the function of nearby cells. DCs play a critical role in responding to and maintaining homeostasis during bacterial infections and disruptions in the skin barrier.

The skin microbiota, consisting of bacteria, fungi, and viruses, protects against invading pathogens physically and provides critical instructions for the innate and adaptive immune responses. Beneficial microorganisms make up the majority of the skin microbiome, however alterations in the commensal-pathogen balance or a breakdown of the microbiota barrier is associated with dermal inflammation. Restoring balance to the microbiome community and reestablishing the barrier, by reducing harmful microorganisms, may suppress cutaneous inflammation.

Accordingly, a Dermal Microbiome-Immunology Assay was conducted to assess the effect of antimicrobial-free **AC Dermal Respiratory Factor Advanced PF** to alter dendritic cell paracrine signaling molecules to kill detrimental microorganisms commonly found on the skin, thereby promoting commensal microbiota.

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Assay Principle

Despite the traditional view of inflammation as 'good' vs. 'bad' and a reduction in the total amount of inflammation is beneficial, the dermal immune system must communicate with the cutaneous microbiome to maintain a healthy balance between commensal and pathogenic microorganisms to prevent excessive dermal inflammation. Table 1 describes the importance of each microorganism tested. This assay examines the ability of immune cell signaling molecules to prevent growth of detrimental microorganisms commonly found on the skin, thus promoting commensal microbiota and reducing downstream inflammation.

Microorganism	Type	Importance
E.coli	Gram-Negative Bacilli (Fermentative)	Human pathogen with multiple antibiotic resistances
S.aureus	Gram-Positive Cocci	Human opportunistic pathogen with antibiotic resistant strains
P.aeruginosa	Gram-Negative Bacilli (Non-Fermentative)	Human opportunistic pathogen and used to study antibiotic resistance and pathogenesis
C.albicans	Yeast	Human opportunistic pathogen responsible for candidiasis
A.brasiliensis	Mold	Human opportunistic pathogen responsible for lung diseases

Table 1. The microorganisms examined and their importance.

Materials

A. Cell Line: Human CD14+ Monocytes (PromoCell, C-12909)

B. Test Organisms: *Escherichia coli* (ATCC #8739); *Staphylococcus aureus* (ATCC #6538); *Pseudomonas aeruginosa* (ATCC #9027); *Candida albicans* (ATCC #10231); *Aspergilus brasiliensis* (ATCC #16404)

C. Incubation Conditions: 37°C at 5% CO_2 and 95% Relative Humidity (RH)

D. Equipment: Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes;

E. Media/Buffers: Dendritic Cell Generation Medium (Complete Media) (PromoCell, C-28050); Cytokine Pack Component A (PromoCell, C-28050); Cytokine Pack Component B (PromoCell, C-28050)

F. Reagents: Sodium Chloride-Peptone Solution pH 7.0; Trypticase Soybean Agar (TSA); Sabouraud Dextrose Agar (SDA)

G. Culture Vessels: T75 tissue culture-treated flask; Fibronectin 6-well clear flat bottom tissue culture-treated plate (Corning, 354402); Petri Dishes (100 x 15 mm)

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Methods

Monocytes were differentiated into DCs according to the manufacturer's protocol and then cryopreserved. To generate conditioned media, DCs were thawed and cultured in a flask with complete media (CM) for 24 hours to ensure cell viability. The cells were then harvested, resuspended in 2 mL of CM, and seeded in a 6-well fibronectin plate. After allowing the DCs to attach for 24 hours, the CM was replaced with 5 mL of either CM (control) or 0.01%, 0.1%, and 1.0% concentrations of antimicrobial-free **AC Dermal Respiratory Factor Advanced PF** diluted with CM. **AC Dermal Respiratory Factor Advanced PF** was tested without Lactobacillus Ferment as it is an antimicrobial. Importantly, the Dendritic Cell Generation Medium is free of antimicrobial agents. After exposing the DCs to experimental conditions for 24 hours, the conditioned media was harvested and placed at -80°C until the Time Kill Test.

For the Time Kill Test, conditioned media samples were thawed and each condition was added into five individual containers. Each container was inoculated with one of the five test organisms. The inoculum concentration for each organism was standardized using the 0.5 McFarland turbidity standard and further diluted to yield approximately 10^6 microorganisms/mL. The amount of each inoculum added to each sample was no more than 1% of the product volume, as to not alter composition. Serial dilutions from each container were performed to enumerate the surviving microorganisms using the Plate Count Technique. The activity of the test material inoculated was evaluated at specific time intervals of 30 seconds, 1, 5, 10 and 30 minutes after the inoculation to quantitatively determine the number of viable microorganisms remaining after the incubation time. Mold and yeast results were read 5 days after plating, and bacteria results were read 2 days after plating.

Area under the curve (AUC) was calculated by adding the areas under the line between each consecutive absorbance measurement using the following equation:

$$AUC = \frac{1}{2}\sum_{i=0}^{n-1}(t_{i+1} - t_i)\,(TKR_i + TKR_{i+1})$$

For Time Kill Results values *TKR1* and *TKR2* at times *t1* and *t2*, the AUC between those two time points is equivalent to the product of difference in time and the average of the two Time Kill Results values.

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Results

The data obtained from this study met criteria for a valid assay as the control performed as anticipated across all five microorganisms. DCs incubated with antimicrobial-free **AC Dermal Respiratory Factor Advanced PF** at 0.01%, 0.1%, and 1.0% prevented growth in all microorganisms tested within 30 seconds.

		TIME KILL TEST RESULTS					
Microorganism	Sample	Inoculum Concentration	30 seconds	1 minute	5 minutes	10 minutes	30 minutes
E.coli	CM (Control)	2.4×10^6	77.2%	77.6%	79.5%	82.3%	87.5%
	0.01% **20219PF**	2.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
	0.1% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
	1.0% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
S.aureus	CM (Control)	2.3×10^6	83.5%	84.2%	88.2%	88.5%	88.7%
	0.01% **20219PF**	5.3×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
	0.1% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
	1.0% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
P.aeruginosa	CM (Control)	1.0×10^6	80.3%	81.3%	81.9%	82.0%	82.0%
	0.01% **20219PF**	3.2×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
	0.1% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
	1.0% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
C.albicans	CM (Control)	5.0×10^5	78.6%	79.0%	79.5%	80.1%	80.5%
	0.01% **20219PF**	1.4×10^5	99.9%	99.9%	99.9%	99.9%	99.9%
	0.1% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
	1.0% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
A.brasiliensis	CM (Control)	1.4×10^5	80.1%	79.3%	78.5%	75.2%	70.9%
	0.01% **20219PF**	1.0×10^6	99.9%	99.9%	99.9%	99.9%	99.9%
	0.1% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%
	1.0% **20219PF**		99.9%	99.9%	99.9%	99.9%	99.9%

Table 2: Time Kill Test results for dendritic cells incubated with antimicrobial-free **AC Dermal Respiratory Factor Advanced PF (20219PF)** inoculated with the tested microorganism populations. Results demonstrate a percent reduction in viable organisms after inoculation and sampling time intervals.

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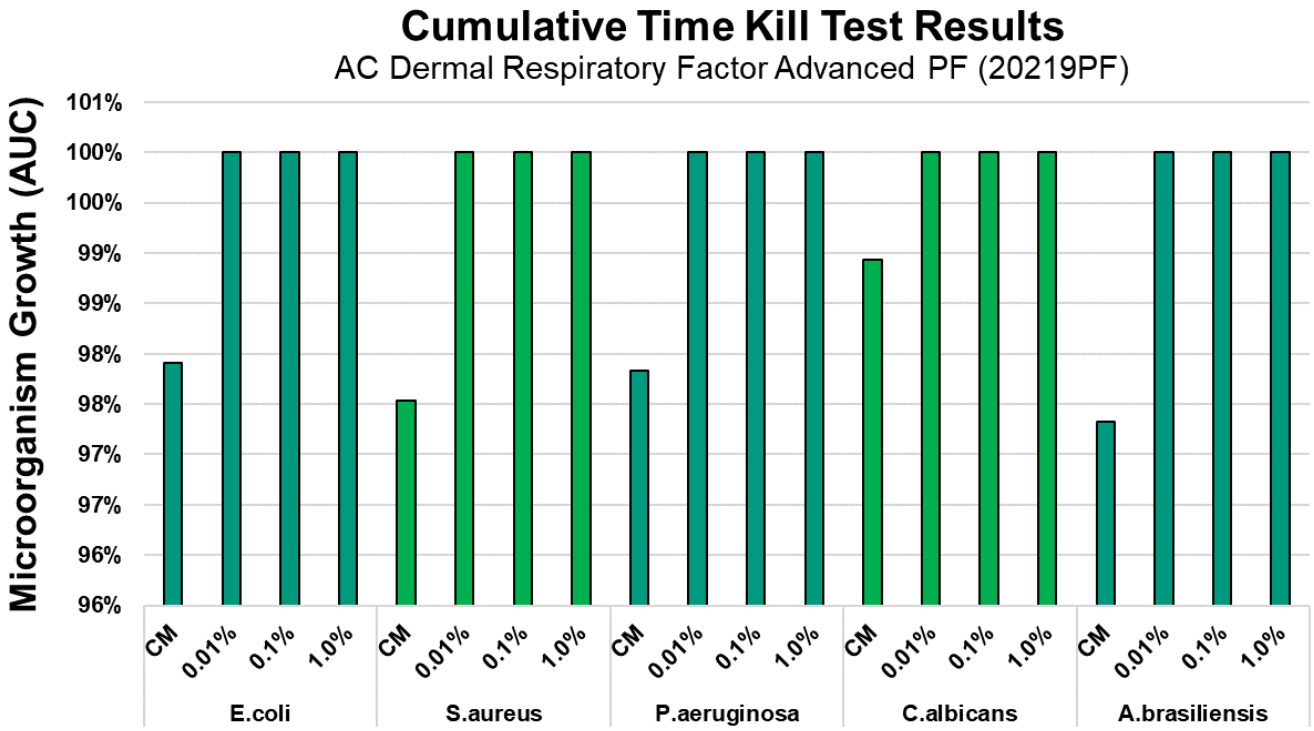

Figure 1: Time Kill Test results for dendritic cells treated with antimicrobial-free **AC Dermal Respiratory Factor Advanced PF** inoculated with the tested microorganism populations across time intervals. Values indicate the percent of microorganisms killed.

Discussion

As shown in Table 2 and Figure 1, the signaling molecules from untreated DCs were unable to kill 100% all microorganisms tested at any time interval. Cumulatively, the media from untreated DCs killed 98%, 98%, 98%, 99%, and 97% of the microorganisms for *E.coli*, *S.aureus*, *P.aeruginosa*, *C.albicans*, and *A.brasiliensis*, respectively. These data demonstrate the signaling molecules from untreated DCs allowed growth of the microorganisms tested.

Conversely, the signaling molecules from DCs treated with antimicrobial-free **AC Dermal Respiratory Factor Advanced PF** at 0.01%, 0.1%, and 1.0% prevented growth at every time point in *E.coli*, *S.aureus*, *P.aeruginosa*, *C.albicans*, and *A.brasiliensis*. The data demonstrate the paracrine signaling molecules from DCs exposed to antimicrobial-free **AC Dermal Respiratory Factor Advanced PF** killed the growth of all microorganisms tested within 30 seconds.

DCs respond to and maintain skin homeostasis during bacterial infections and disruptions in the skin barrier through paracrine signaling molecules. Specifically, DCs can limit excessive inflammation by restoring balance to the dermal microbiome community and reestablishing the physical barrier. These data indicate **AC Dermal Respiratory Factor Advanced PF** augmented the ability of signaling molecules from DCs to kill detrimental microorganisms commonly found on the skin, thus promoting commensal microbiota.