

130 Erick Street  
Crystal Lake, IL 60014  
(815) 526-0954

Final Report for:  
Attn: Oscar the Grouch  
160 Sesame Street  
TV Land, USA 00000

Test Method:  
MIL STD 810F Method 508.5 Fungus

MSL Project # R2011-XXX

Sample Received: 11/8/11

Testing Initiated: 11/14/11

Testing Completed: 12/12/11

Report Issued: 12/15/11

Analyst: *Agata Shulfer*  
Title: Operations Manager

Approved by: *Judy LaZonby*  
Title: Laboratory Director





**Objective:**

The purpose of this test is to assess the extent to which materiel will support fungal growth.

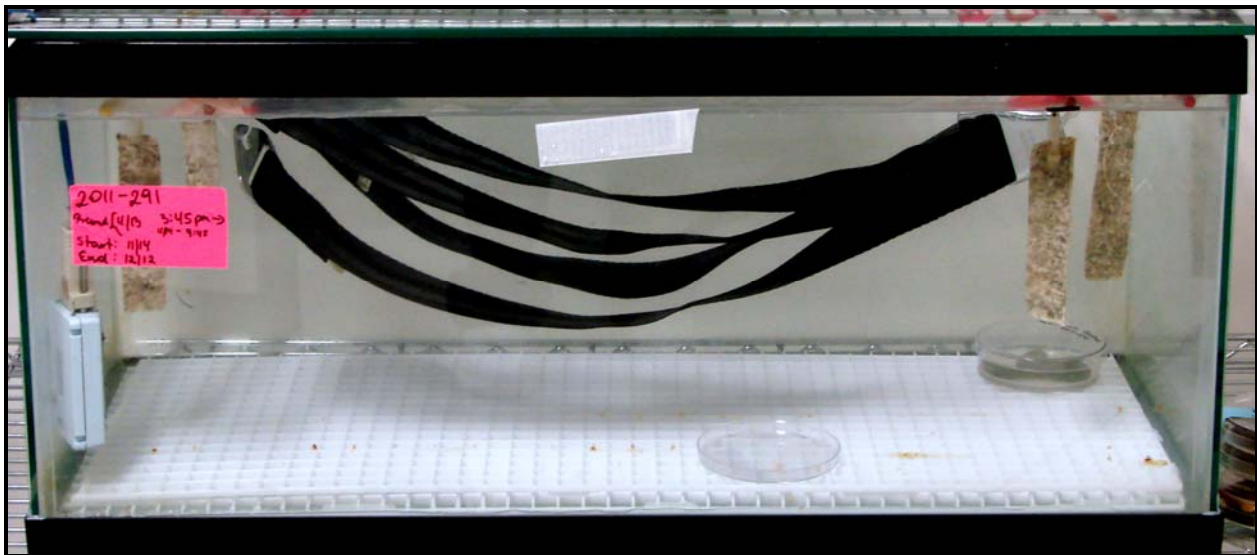
**Pre-test Documentation:**

Two-piece safety restraining belt was submitted for testing. The UUT's size was approximately 2" by 20" by 1" in size. The sample pieces were black with metal parts on both ends. The UUT's were identified as followed:

1. Belt, Tongue; XXX-02-11-00-2-2; Lot: 510000001
2. Belt, Buckle; XXX-02-11-00-2-25; Lot: 510000007

The test sequence, environmental test history, or pre-test operations were not specified prior to testing. Details are described below. Testing followed the MIL STD 810F method 508.5 protocol. This is fungus method indicated in the Test Plan X-00i Garbage Can Armor Protection System (APS) Climactic Qualification Doc. No. GCA-CQ-484 protocol.

The test chamber was a glass aquarium approximately 30" x 12" x 13" in size fitted with a glass top. The bottom of the tank contained approximately 1 inch of distilled water to maintain humidity. Above the water a grate was placed to protect the test piece or controls from inadvertently falling into the water at any point during testing. The test piece and controls were suspended using clips and cable ties attached to rods. Prior to testing the chamber was decontaminated using hot water and a quaternary amine microbial decontaminant cleaner.



**Test Chamber**

All test pieces were cleaned prior to testing with reagent grade isopropyl alcohol. All cleaned test pieces were allowed to air dry for 72 hours before proceeding with the testing.





The inoculum was prepared from pure fungal stock cultures incubated at  $30 \pm 1^\circ\text{C}$  for 10 to 21 days. The following U.S. group test fungi were used:

1. *Aspergillus flavus* ATCC 9643
2. *Aspergillus versicolor* ATCC 11 730
3. *Penicillium funiculosum* ATCC 11 797
4. *Chaetomium globosum* ATCC 6205
5. *Aspergillus brasiliensis*<sup>1</sup> ATCC 9642

<sup>1</sup>*Aspergillus niger* has been reclassified as *Aspergillus brasiliensis* but maintained its original ATCC number.

Spore suspensions containing  $1,000,000 \pm 20\%$  spores per milliliter as determined with a counting chamber were prepared for each organism. The viability of the individual spore suspensions were verified by inoculating the entire surface of Potato Dextrose Agar plates and checking for growth after 7 to 10 days incubation at  $30 \pm 1^\circ\text{C}$ . See results below in Table #1. The control checks of the viability of the individual spore suspensions were run concurrently with the testing. Equal volumes of the individual fungal cultures were blended to obtain the mixed spore suspension to be used for inoculation of test items. A viability control of the mixed inoculum was prepared by placing sterile filter paper on mineral salt agar and inoculating these with the spore suspension using a sterile atomizer. See results below.

Control strips of unbleached, plain weave cotton cloth cut into 3 cm X 4 inch strips were dipped into the solution described within the method and allowed to dry. These strips were hung within the chamber close to and bracketing test items to ensure the test strips and test items experience the same test environment. The test pieces and control strips were held in the test chamber to precondition to  $30 \pm 1^\circ\text{C}$  and a relative humidity of greater than 90% and less than 100% for a minimum of 4 hours prior to inoculation.

Each test item and control strip was inoculated with the mixed spore suspension by spraying the items with a fine mist from a sterile atomizer. The items were covered completely with the spore suspension on both sides, spraying until drops began to form on the surface. Immediately after spraying, the test items and control strips were suspended from rods in the test chamber. The test chamber contained water to maintain the desired relative humidity required by the test method of greater than 90% and less than 100%. The temperature within the test chamber was maintained at  $30 \pm 1^\circ\text{C}$  for the duration of testing as required by the test method.

Relative humidity and temperature are monitored using the following equipment:

- Relative humidity is validated using a Vaisala Humidity and Temperature Meter and Probe MI70/HMP75B. The relative humidity reading of the equipment is internally validated to NIST traceable standards using  $\text{K}_2\text{SO}_4$  saturated salts, FINAS Certificate of Calibration #K008-U00038.
- Temperature is validated using a Vaisala Humidity and Temperature Meter and Probe MI70/HMP75. Temperature equipment is internally validated to NIST traceable standards using an externally calibrated Cole Palmer Thermometer, Serial #4463; A2LA accredited ISO 17025 Cert. #1746.01.





- Veriteq Data Logger, NIST traceable certificate #0147202, Serial #09102066. See Table # 2 below for weekly validations of the Veriteq Datalogger V#8.






After 7 days, the growth on the control strips was inspected. The control strips were checked again at the end of testing for an increase in fungal growth. See results below.

Provided the control strips and viability of spore suspensions were acceptable, the test was continued for 28 days incubation. At the end of the incubation, the samples were examined for fungal growth. Results are described in the Post Test Documentation section below. The assigned ratings were determined using the rating scheme in Table #3 that is listed in the method.

**During Test Documentation:**

Incubation began on 11/14/11 at 10:00 a.m.

**Table -1 Viability of Individual Spore Suspensions**

Organism	Percent Coverage	Viability Plate at 7 Days Incubation	
<i>Aspergillus brasiliensis</i> ATCC # 9642	100%		
<i>Aspergillus flavus</i> ATCC # 9643	100%		
<i>Aspergillus versicolor</i> ATCC # 11730	100%		
<i>Penicillium funiculosum</i> ATCC # 11797	100%		
<i>Chaetomium globosum</i> ATCC # 6205	100%		





Temperature and relative humidity were maintained throughout the entire test period. See Table # 2 for weekly validations of the Veriteq Datalogger V# 8.

**Table #2 – Record of Critical Components**

Critical Component	Pre-Condition (11/13/11 16:00 -9:45 11/14/11)	Week 1 (11/21/11)	Week 2 (11/28/11)	Week 3 (12/5/11)	Week 4 (12/12/11)
Temperature (30 ± 1°C)	30.0	29.7	29.6	29.6	29.5
Humidity (>90% and<100%)	93.6	98.6	97.1	97.4	96.9

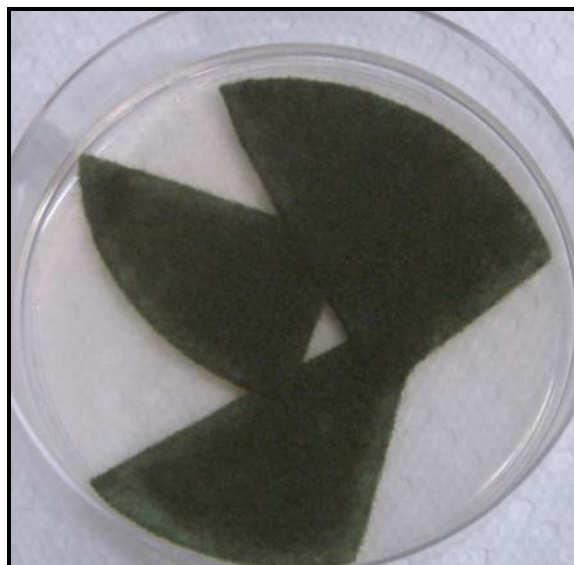
MIL STD 810 F Method 508.5 Critical Component Requirements:

- Temperature cannot exceed 40°C
- Temperature cannot exceed 32°C for 4 hours or more
- Temperature cannot go below 28°C and have a drop in humidity to less than 90%.
- If temperature does fall below the test parameters (29°C) but humidity has been maintained at 90% or greater, reestablish test conditions and continue test at the point the test fell below the prescribed tolerances.
- Relative humidity cannot drop below 50%
- Relative humidity cannot drop below 70% for 4 or more hours
- If there is evidence of deterioration of fungal growth on the control strips that may be due to test interruptions which affected the temperature and humidity, the test must be restarted.



The picture on the left is a chamber control strip at Day 0. The picture on the right is a chamber control strip at Day 28. At Day 7, all control strips had acceptable fungal growth to confirm the viability of the spore suspension and that the environment was suitable for fungal growth. At Day 28, all chamber controls had an increase in fungal growth as compared to Day 7 as required by the test method. All chamber controls performed as expected confirming the validity of the test.





**Mixed Inoculum Viability Control**

Incubation ended on 12/12/11 at 10:00 a.m. at which time test items were examined for fungal growth.

**Post Test Documentation:**

Two UUT's and four control samples were tested for 28 days. The testing was performed without interruption. Performance data was not done.

Upon removal of the chamber at Day 28, the test pieces were evaluated following the rating scheme listed below in Table #3. Test pieces were first examined with an unaided eye and then more closely inspected with a stereoscope. Any possible fungal growth was examined by tape preparation and microscopic evaluation. Fungal growth was determined to be test organisms or non-test organisms. Since samples are not sterile prior to testing, it is common for non-test organisms that are native to the test samples to appear. See Table # 4 for results details.

**Table # 3 – Evaluation Scheme for Visible Effects and Test Sample Ratings**

Amount of Growth	Rating	Comments
None	0	Substrate devoid of microbial growth
Trace	1	Scattered, sparse or very restricted microbial growth
Light	2	Intermittent infestations or loosely spread microbial colonies on substrate surface. Includes continuous filamentous growth extending over the entire surface, but underlying surfaces are still visible
Medium	3	Substantial amount of microbial growth. Substrate may exhibit visible structural change
Heavy	4	Massive microbial growth





## Results

**Table # 4 - Samples Rating after 28 Days Incubation**

Sample		Grade	Description of Growth
Belt, Tongue; XXX-02-11-00-2-2; Lot: 510000001	Fabric	1	Trace amount of fungal growth was found all over the tested sample surface. The growth was more easily seen on the fabric stitching. Test organisms found on this sample: <i>A. flavus</i> , <i>A. niger</i> , <i>A. versicolor</i> . The growth was generally visible with unaided eye.
	Metal parts	0	No fungal growth was found on the metal parts of this sample. Substrate devoid of microbial growth.
Belt, Buckle; XXX-02-11-00-2-25; Lot: 510000007	Fabric	1	Trace amount of fungal growth was found all over the tested sample surface. The growth was more visible on the fabric stitching. Test organisms found on this sample: <i>A. flavus</i> , <i>P. funiculosum</i> , <i>A. versicolor</i> . The growth was generally visible with unaided eye.
	Metal parts	0	No fungal growth was found on the metal parts of this sample. Substrate devoid of microbial growth. Slight staining/rusting was noticed on one of the metal pieces of this sample





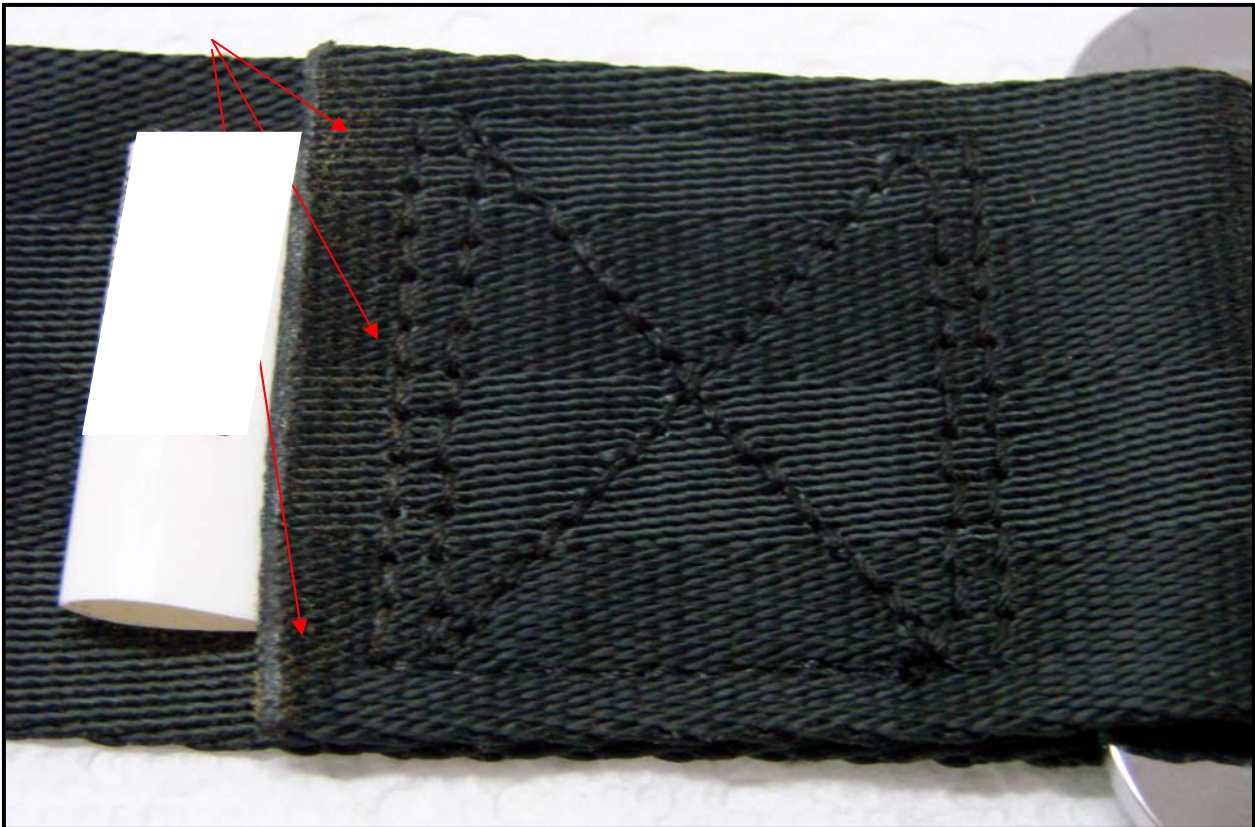
**Belt, Tongue; XXX-02-11-00-2-2; Lot: 510000001 - Before inoculation**





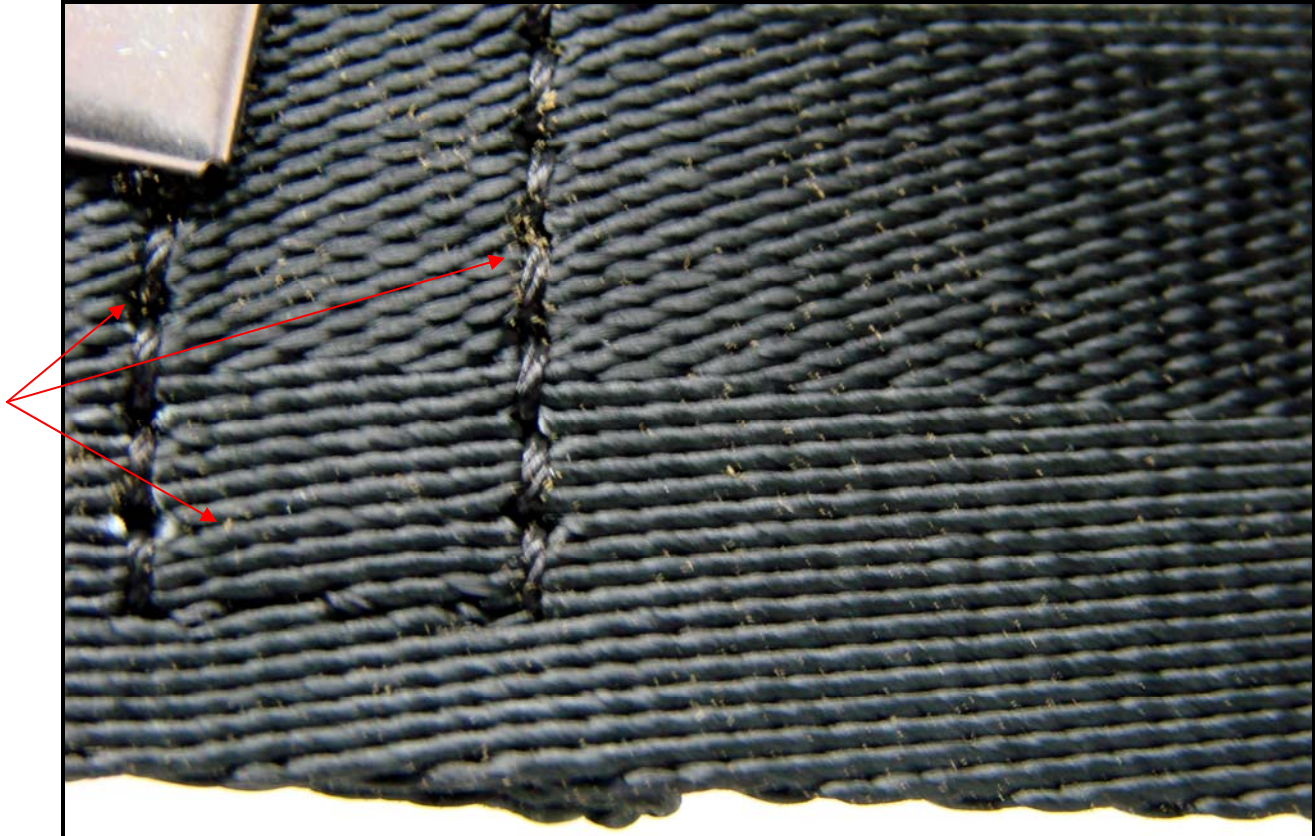


**Belt, Tongue; XXX-02-11-00-2-2; Lot: 510000001 - After 28 days incubation**



The fungal growth found on this sample was present only on the fabric; no fungal growth was found on the metal parts of this sample.





In a close-up view, the trace amount of fungal growth was found all over this sample but was more visible around the stitching of the belt (indicated by the arrows above).



*A. flavus* was found all over the fabric surface, as shown in this photomicrograph taken from a tape lift from the sample surface.



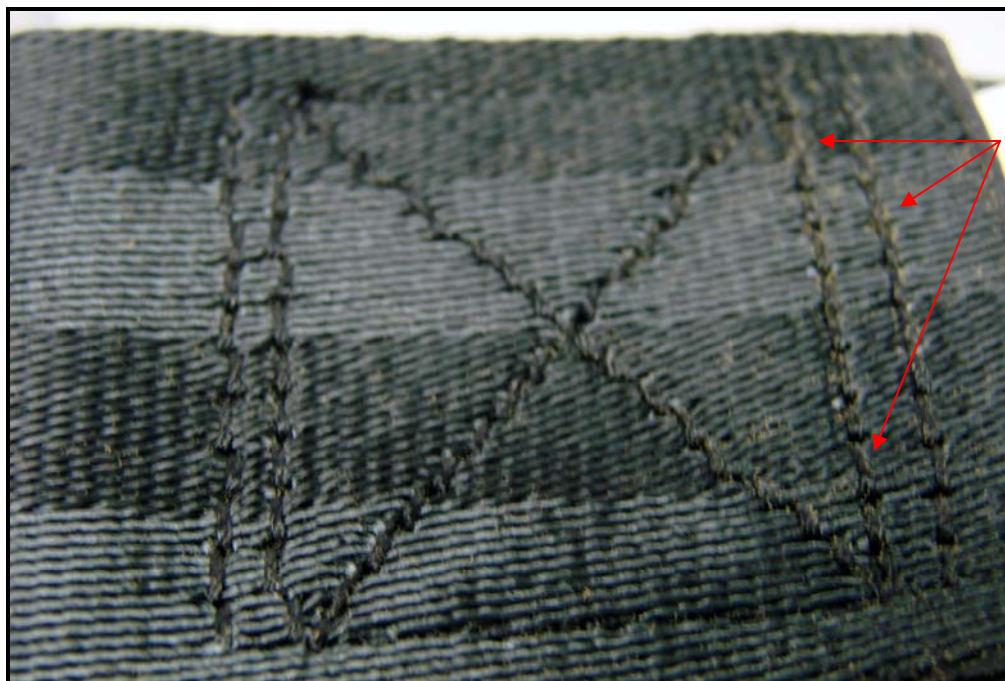


**Belt, Buckle; XXX-02-11-00-2-25; Lot: 510000007- Before inoculation**

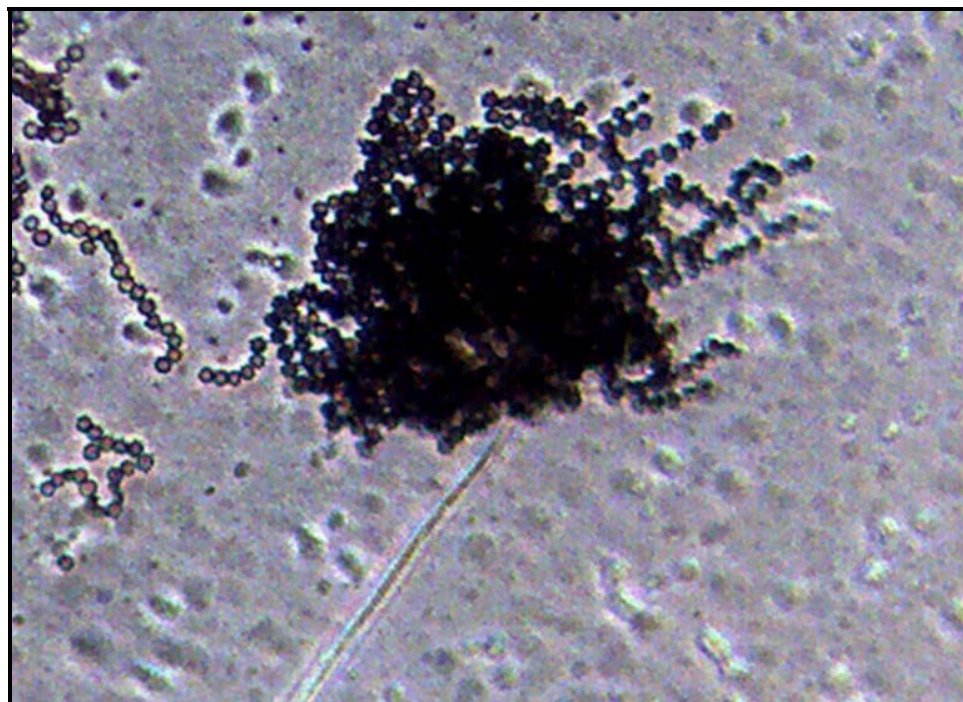


**Belt, Buckle; XXX-02-11-00-2-25; Lot: 510000007 - After 28 days incubation**





The trace amount of fungal growth found on this sample was mostly visible around the stitching of the belt (indicated by the arrows above).



This *Aspergillus niger* was found on all the fabric from both tested samples.





The trace amount of fungal growth was found all over the fabric part of the sample, the growth was short, thin and scattered all over the tested surface.



This metal piece from sample: Belt, Buckle; 682-42-13-01-1-1; Lot: 51002600 had slight discoloration/rusting present after 28 days incubation.





## Conclusions

- The tested UUT's had a trace amount of fungal growth after the 28-day incubation. Only test organisms were recovered on the tested pieces after the incubation. These results are recorded in the Post-Test Documentation Section.
- The amount of fungal growth shown in the pictures occurred over the course of 28 days. When the control strips were checked for growth after 7 days, the test pieces were only inspected through the glass within the chamber with the unaided eye. At that 7-day point, all tested samples had trace amounts of visible growth. Descriptions of the Day 28 growth are given in the Post-Test Documentation Section.
- The fungal growth did not appear to affect the integrity of the surface of the samples. Slight discoloration/rusting on one of the metal pieces from UUT: Belt, Buckle; 682-42-13-01-1-1; Lot: 51002600, was noted. Physical interference and detrimental effects on performance were not evaluated in the mycology lab. Any item with fungal growth may pose a health risk for persons with allergies to mold.
- The fungal growth appeared such that wiping of the surface could partially remove the growth from site but not completely eliminate the fungus from the test pieces. Aesthetically, most of the fungal growth was not visible with the unaided eye.

## Equipment List

Instrument	Make	Model	MSL Asset #	Serial #	Measurement/ Performance Documentation	Year Manufacture
Autoclave	Hirayama HICLAVE	HVE-50	MSL-AUTO1	30608072451	Time and temperature verified during each run using autoclave data logger.	Sept. 08
Autoclave	Sanyo	Mac 1200	MSL-Auto2	71765		NA
Autoclave Temperature Data logger	ACR Nautilus	135	MSL-1007	11818	Validated against reference standard MSL-0954. Last validation 9/23/11.	NA
Autoclave Temperature Data Logger	ACR Nautilus	135	MSL-0954	11672	Reference Standard externally calibrated 11/25/10 to NIST traceable standards by A2LA accredited ISO/IEC 17025:2005 – Calibration Cert. #2260.01.	NA
Balance	Adam Equipment	Highland HCB 123	MSL-0973	AE75900492	Daily internal validations preformed using MSL-100	NA
Weight Set	Denver Instruments	100g-10mg Class 1	MSL-100	07-81869	Validated against reference standard MSL-105. Last validation done 9/20/11. Internal validation was also conducted on 9/20/11 by inter-weight set comparison.	Nov. 06
Weight Set	Troemner	Class 1 SS	MSL-105	71722	Reference standard externally calibrated 6/21/10 to NIST traceable standards by NVLAP accredited ISO/IEC 17025 Laboratory -Code#105013	NA





Instrument	Make	Model	MSL Asset #	Serial #	Measurement/ Performance Documentation	Year Manufacture
pH electrode	Accument	Spear tip 13-620-133	MSL-1008	562911	These instruments are used together working as one instrument. pH meter and probe validated internally daily with NIST traceable pH standard buffers. Meter and probe standardized internally on 6/21/11 using NIST traceable pH Singles standards.	NA
pH Meter	Denver Instruments	Ultra Basic UB-10	MSL-0996	UB10096236		
Data Logger Temperature and Relative Humidity Sensor	Veriteq	SP-2000-20R	V8	09102066	Internal validation using MSL-Vaisala1 performed: 11/14/11.	
Thermo-hygrometer	Vaisala	MI70, HMP75B	MSL-Vaisala1	D2050019, F1660002	Externally calibrated for temperature and humidity on 3/17/11 to NIST traceable standards by Vaisala. RH internally validated 9/22/11 against reference standard K <sub>2</sub> SO <sub>4</sub> saturated salt solution calibrated by a FINAS accredited ISO 17025 Cert. No. K008-U00038. Temperature internally validated on 9/22/11 using MSL-ThermRef.	NA
Thermometer	Fisherbrand/ Ertco	Glass -1 to 101 degrees C	MSL-Therm Ref	1337	Internally validated 9/7/11 against MSL-ThermRef2	NA
Thermometer	Cole Palmer	90250-31	MSL-Therm Ref2	4463	Reference Standard externally calibrated to NIST traceable standards 5/27/11 by A2LA accredited ISO 17025:2005 Calibration Cert. # 1746.01	NA
Hemocytometer	Brightline	NA	MSL-0960	NA	NA	NA
Hemocytometer	Spencer Brightline	NA	MSL-0959	NA	NA	NA
Hemocytometer Cover Slips	Fisher Scientific	#2026H	MSL-1021	Lot #031011-7	NA	NA
Air Pump	Barnet Air Cadet 420-3901	420-3901	MSL-0967	G08002770	NA	July 08
Atomizer	Chemglass	125 mL	NA	NA	NA	NA
Environmental Test Chamber	MSL	NA	MSL-012	NA	NA	NA

