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04/21/2011
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Candida Morphology: Presence of Pseudohyphae Used in the Early Identification of *Candida glabrata* versus Other *Candida* Species

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Abstract

Candida Morphology: Presence of Pseudohyphae Used in the Early Identification of *Candida glabrata* versus Other *Candida* Species

By Gwen M. Abdulhafid

Fungal infections caused by *Candida* species are the fourth leading cause of nosocomial bloodstream infections in the United States (CDC, 2008). Although *Candida albicans* is the most commonly isolated species, there has been an increase in the number of non-albicans species isolated. Bloodstream infections caused by *Candida glabrata* are of particular concern because of high mortality rates and growing resistance to fluconazole, the most commonly prescribed medication for *Candida* infections. Inappropriate treatment, such as treating a resistant organism with fluconazole, or delay of appropriate treatment of *Candida* blood stream infections has been associated with increased costs and increased mortality (Ferguson, nd). Due to the increasing resistance to fluconazole shown by *C. glabrata* and the time required for species to be indentified in standard blood cultures, blood stream infections caused by this species of *Candida* are especially at risk for inappropriate or delayed treatment. A unique characteristic of *C.* glabrata is that it does not form pseudohyphae. The primary objective of this research was to analyze the data gathered at the initial identification of a blood culture positive for yeast and compare it to the final culture report to assess the predictive value of the presence or absence of pseudohyphae in identifying *Candida* species, in particular *C. glabrata*. Secondary objectives included identifying factors that may influence the primary objective. Data was collected for a year at Emory Healthcare facilities. 348 yeast cultures from 145 patients were assessed. **Results:** Initial analysis of the data indicated that pseudohyphae were not noted for any culture positive for *C. glabrata,* a 100% negative predictive value. The risk for *C. glabrata* in a yeast culture without pseudohyphae that grows in an anaerobic bottle, controlling for hours to positive and facility, was determined to be more than 3 times that of other *Candida* species (RR=3.09, CI 2.02, 4.72, $p < .0001$). Alternatively, the risk for *C. Glabrata* in a yeast culture without pseudohyphae that takes greater than 36 hours to become positive, controlling for aerobic status and facility, is almost twice that of other *Candida* species (RR =1.86, CI 1.18, 2.95, $p = .008$).

Candida Morphology: Presence of Pseudohyphae Used in the Early Identification of *Candida glabrata* versus Other *Candida* Species

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Introduction

Fungal infections caused by *Candida* species are the fourth leading cause of nosocomial bloodstream infections in the United States (Centers for Disease Control and Prevention [CDC], 2008). Although *Candida albicans* is the most commonly isolated species, there has recently been an increase in the number of non-albicans species isolated. Bloodstream infections caused by *Candida glabrata* are of particular concern because of high mortality rates and growing resistance to fluconazole, the most commonly prescribed medication to treat *Candida* infections.

Nosocomial infections delay clinical improvement, prolong hospitalization, increase healthcare costs, and increase mortality rates. The overall mortality rate for patients admitted to acute care hospitals in the United States is 2.5% (Gudlaugsson et al. 2003). In comparison, studies have reported crude mortality rates of 40-75% for patients with nosocomial candidemia (Gudlaugsson et al. 2003); mortality rates after ruling out other causes range from 5-71% (Falagas, Apostolou and Pappas, 2006). A recent matched case control study by investigators at the University of Iowa hospital reported a 61% crude mortality rate in candidemia cases. The crude mortality rate in control cases was 12%, resulting in a mortality rate difference of 49%. (Gudlaugsson, 2003). The Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) surveillance system of nosocomial bloodstream infections in U.S. hospitals reports a 40% crude mortality rate and a 25% mortality rate for patients with *Candida* bloodstream infections after ruling out other causes (Wenzel and Edmund, 2001). The highest mortality rates have been reported for infections caused by non-albicans species, with *C. glabrata* having the highest mortality rates (Picazo et al. 2008).

Historically, more than 75% of all *Candida* bloodstream infections were caused by *Candida albicans.* Widespread empiric and prophylactic use of fluconazole has resulted in the development of resistant species. Recently there has been a significant shift toward non-albicans species causing nosocomial infections, with estimates as high as 50% of all the *Candida* infections (Horvath et al. 2007). Incidence rates for *C. glabrata* bloodstream infections are increasing accordingly.

Fluconazole has long been the treatment of choice for candidemia infections. Standard treatment for a *Candida* bloodstream infection is 200-400 mg. of fluconazole daily for 14 days after first negative blood culture; oral dosing is preferable and less costly. *C. glabrata,* however, has a growing association with fluconazole resistance. Lee et al. (2009) determined previous use of fluconazole for treatment or prophylaxis is a significant risk factor for fluconzole-resistant *C. glabrata* bloodstream infection. Other risk factors for candidemia include use of broad spectrum antibiotics, central venous access, corticosteroid or immunosuppressive use, total parenteral nutrition, dialysis, diabetes, abdominal surgery, and high severity of illness (Amrutker et al. 2006, Lee et al. 2009, Playford et al. 2008, Tumbarello et al. 2009). As patients with these risk factors live longer and are hospitalized more often, it is likely that the frequency of nosocomial *candida* infections will increase. If fluconazole use increases as well, a consequential rise in the incidence of *C. glabrata* resistance may follow.

Data collected for Emory University Hospital and Emory Midtown Hospital indicates that in 2007 and 2008 *Candida glabrata* caused 39% of *Candida* bloodstream infections. During the period from September, 2006 through September, 2008 approximately 34% of patients with

invasive candidiasis at these two institutions died or were discharged to hospice within 12 weeks of diagnosis.

In recent years, another class of antifungal medications has come to market, echinocandins. The earliest echinocandin was caspofungin. New echinocandins with fewer side effects, micafungin and anidulafungin, have been developed more recently, earning Food and Drug Adminstration (FDA) approval within the last five years. *C. glabrata* has been susceptible to all the echinocandins. However, echinocandins are more costly than fluconazole and some practitioners worry that increased use of echinocandins will hasten development of echinocandin resistant organisms. One species of *candida*, *C. parapsilosis*, is less susceptible to echinocandins than other species, which provides another reason to identify *candida* species as quickly as possible so that appropriate treatment can be initiated.

Problem Statement

Inappropriate treatment, such as treating a resistant organism with fluconazole, or delay of appropriate treatment of *Candida* blood stream infections has been associated with increased costs and, in some studies, with increased mortality (Ferguson, 2009). Due to the increasing resistance to fluconazole shown by *C. glabrata* and the time required for species to be indentified in standard blood cultures, blood stream infections caused by this species of *Candida* are especially at risk for inappropriate or delayed treatment. A case control study using data from the Prospective Antifungal Therapy Alliance registry of invasive fungal infections confirms that time to receipt of adequate therapy was significantly longer for patients with *C. glabrata* bloodstream infections versus those infected with *C. albicans* (Klevay et al. 2009).

Echinocandins are more expensive than fluconazole, an important consideration in this era of rising healthcare costs. The estimated daily cost for fluconazole treatment is $3-$14 for oral treatment and $64-$128 for intravenous (IV) treatment, depending on dose. Caspofungin treatment costs $329-$424 per day, Micafungin and Anidulafungin treatment costs $180-$280 per day (Lewis, 2007). There is no oral formulation of an echinocandin so patients must have IV access to receive the medication. IV access is associated with increased risk of infection and often requires increased length of hospital stay, increasing the indirect cost of treatment. Although treatment with an echinocandin is initially more expensive, it is more cost effective than inappropriate treatment with fluconazole. Treatment that is not wholly effective results in greater health complications for the patient and increased hospital stays. Research comparing costs and length of hospital stay for patients with C. glabrata bloodstream infections versus those with C. albicans bloodstream infections found that both costs and length of hospital stay were significantly higher for patients with C. glabrata: $56,026 vs $32,810; $p = .04$, and 19.7 days vs 14.5 days; $p = .05$ (Moran, et al. 2010).

When a blood culture grows yeast it generally takes 24-96 hours to become positive. It can take an additional 24-72 hours for the species of yeast to be identified. In a worst case scenario, a patient receiving standard treatment for a *Candida* bloodstream infection could be treated with fluconazole for up to six days before it was known that the infection was caused by a fluconazole-resistant species such as *C. glabrata*. Delayed treatment with an effective antifungal is strongly associated with increased mortality. Starting appropriate therapy at diagnosis is associated with a 15% mortality rate. Delaying appropriate therapy by one day increases mortality to 24%. Delaying two days has an associated mortality rate of 37% and delaying three days increases mortality to 41% (Ferguson, 2009)

Objectives

The morphology of *Candida glabrata* (Figure 1) is significantly different than other species of yeast in that it is the only *candida* species that does not develop pseudohyphae. (Fidel et al. 1999)

Figure1: *Candida glabrata* (Courtesy of medmicro.wisc.edu)

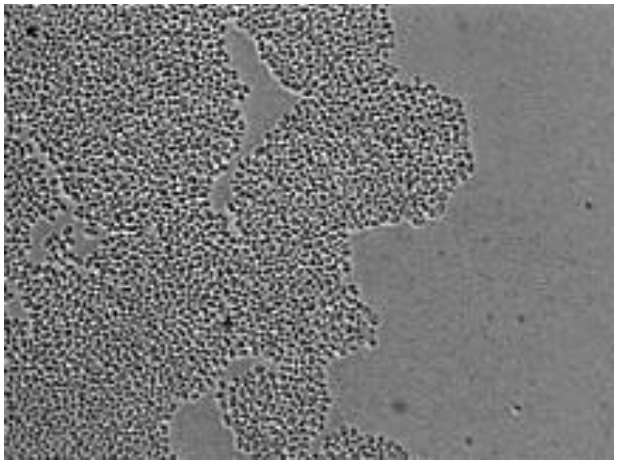
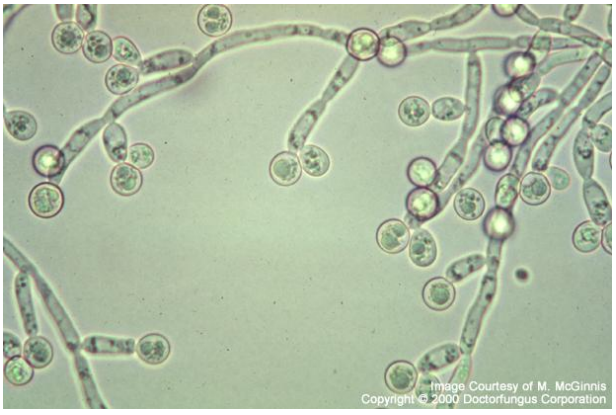


Figure 2: *Candida* with pseudohyphae



Courtesy of M. McGinnis, Dr. Fungus Corporation

At the time of the initial identification of a positive blood culture pseudohyphae may be seen on non-*glabrata candida* species (Figure 2). In an attempt to identify *C. glabrata* infections as quickly and efficiently as possible, the Infectious Diseases division of Emory Healthcare requested that the microbiologist that first notes a positive yeast culture also notes the presence of pseudohyphae if they are identified. If pseudoyphae are not seen, the likelihood of a *C. glabrata* infection is increased and it may be appropriate to start treatment with an echinocandin rather than fluconazole.

The primary objective of this research was to analyze the data gathered at the initial identification of a blood culture positive for yeast and compare it to the final culture report to assess the predictive value of the presence or absence of pseudohyphae in identifying *Candida* species, in particular *C. glabrata* versus species that are not resistant to fluconazole.

Secondary objectives included identifying factors that may influence the primary objective, i.e. time until a culture becomes positive, anaerobic versus aerobic cultures, and the laboratory shift during which the culture was first noted as positive.

Methodology

The Emory microbiology lab uses a Becton Dickinson (BD) BACTEC blood culture system. When a physician suspects a bloodstream infection and orders blood cultures, blood is drawn from the patient through peripheral venipuncture, through a central venous catheter, or from both, and is injected into two blood culture bottles containing culture broth. One bottle is aerobic and the other is anaerobic, allowing for the growth of microbes that grow in either the presence or absence of free oxygen. These bottles are transported to the microbiology lab within an hour of the blood draw and logged into the automated BD microbiology growth and detection

system. The culture bottles are then placed into the culture machine and are allowed to incubate at 35°C.

If microbial growth occurs in a blood culture bottle the production of carbon dioxide increases, which results in a change in pH and a subsequent color change of the growth media. The culture machine automatically scans the bottles with fluorescent light every 15 minutes to detect changes in color. If a change is noted, an alarm sounds alerting lab technicians of the positive culture. Technicians gram stain a sample of the positive culture, observe it under a microscope, and note on a worksheet the type of specimen growing in the sample, i.e. gram negative rods, gram positive cocci, or yeast. It is at this point the lab technicians would note the presence or absence of pseudohyphae if the specimen was yeast. It is also at this point that the patient's nurse is notified that the patient's blood culture is positive for yeast. The nurse then notifies the physician who gives an empirical treatment order and medication therapy is initiated.

After the presumptive identification of a positive sample the microbial specimen is transferred to a dextrose agar plate for additional growth and biochemical analysis. The microbiology lab at Emory uses the API 20C AUX system to identify yeast species. This system analyses the carbohydrate assimilation of the sample to determine species (Shaheen and Taha, 2006). The species identification process generally requires 48 to 72 hours.

Data Collection

Data was obtained from microbiology for the year 2009 regarding the first notation of a blood culture positive for yeast. This data was in the form of a worksheet on which the microbiologist made a handwritten note regarding the positive culture. It is on this worksheet that the presence of pseudohyphae was noted (Appendix A). The identification, or accession,

numbers for positive yeast cultures as well as the patient names noted on the worksheet were used to look up the final culture reports in the patients' medical records.

Once all positive cultures were matched with a patient (patients often have more than one positive culture) study ID numbers replaced patient names and the link between patient name and study ID was destroyed. Other patient data gathered included age, facility (Emory Hospital, Emory Midtown, or Wesley Woods), and date of death if death occurred within 12 weeks of positive culture. Culture data collected included date and time of a patient's first blood culture positive for *Candida* and dates of any subsequent blood cultures positive for *Candida*, dates and times of microbiology reports identifying *Candida* species, hours to positive (time between blood draw and initial microbial growth), the shift the culture was identified as positive, and whether or not the culture grew in an aerobic or anaerobic bottle, or both.

Data Analysis

Initial analysis of the data indicated that pseudohyphae were not noted for any culture positive for *C. glabrata*, a 100% negative predictive value (Appendix B). Subsequent univariate and multivariable analysis was performed using data only from those cultures that did not have pseudohyphae noted. It was assumed that in addition to those worksheet entries that noted "no pseudohyphae" those without a notation (n = 244) at all were negative for pseudohyphae presence as well; only a specific reference to the presence of pseudohyphae was considered a positive response.

Not all cultures had data available to assess hours to positive. Using available data, the mean hours to positive was calculated for each species and missing values were assigned the

mean for that species. This method was compared to an analysis using only those cultures with an hour to positive value. The results were similar with the larger data set (with missing values replaced by the mean) producing somewhat more conservative risk estimates.

Aerobic status was considered a dichotomous variable for the purpose of analysis; a culture was either anaerobic or not anaerobic. Those cultures that grew in an anaerobic bottle, whether they grew in only an anaerobic bottle or in both the aerobic and anaerobic bottles were designated “anaerobic”.

Regression analysis considering aerobic status the primary exposure variable used hours to positive in the model as a continuous variable. Because aerobic status is not always immediately available to the treating physician an additional analysis, using hours to positive as the primary exposure variable was also done. In this instance hours to positive was considered a dichotomous variable with the values of greater than 36 hours or less than or equal to 36 hours.

Univariate analysis was performed using ANOVA and Chi Square tests in SAS® 9.2 (Cary, N.C., 2010). Those variables with a significant p-value ($p<.05$) were included in the logistic regression model. All confidence intervals were estimated using Wald 95% confidence limit analysis. As the rare disease criterion does not apply to the sample in this retrospective cohort study, the PROC GENMOD command in SAS was used to estimate risk ratios. Descriptive data analysis was performed using Microsoft Excel (2007) and SAS. Positive and negative predictive values were determined using OpenEpi© version 2.3.1 (2010).

Results

A total of 348 blood cultures from 145 patients were found to be positive for *Candida.* Of these, 65 were noted to have pseudohyphae present. Mean age of patients was 57.9 years. There were 118 cultures (34%) positive for *C. glabrata.* Nine species of *Candida* were ultimately identified (Table 1). The mean hours to positive for all species combined was 45.9 (Figure 3). At 57.6 hours the mean hours to positive was highest for *C. glabrata* (Table 1). Some specimens grew in anaerobic bottles only (n=25) and 115 grew in both aerobic and anaerobic bottles. Over half the specimens (n=183) grew only in the aerobic bottle. For reasons unknown a few specimens (n=13) were incubated only in the aerobic bottle and 12 cultures did not have aerobic status noted. A total of 193 yeast cultures were obtained from Emory University Hospital, and 113 were from Emory Midtown Hospital, the remainder was from other locations associated with Emory Healthcare (Table 2). It is interesting to note that hours to positive was significantly longer ($p = .03$) for cultures from Emory Midtown (53.6 hours) than for those from Emory University Hospital (43.4 hours). There was a fairly equal distribution of positive cultures across all three shifts. The number of patients who are known to have died within 12 weeks of a positive yeast culture equaled 50; the status of 13 patients is unknown.

Figure 3: Distribution of time to positive for all blood cultures positive for *Candida* collected at Emory Healthcare associated facilities in 2009.

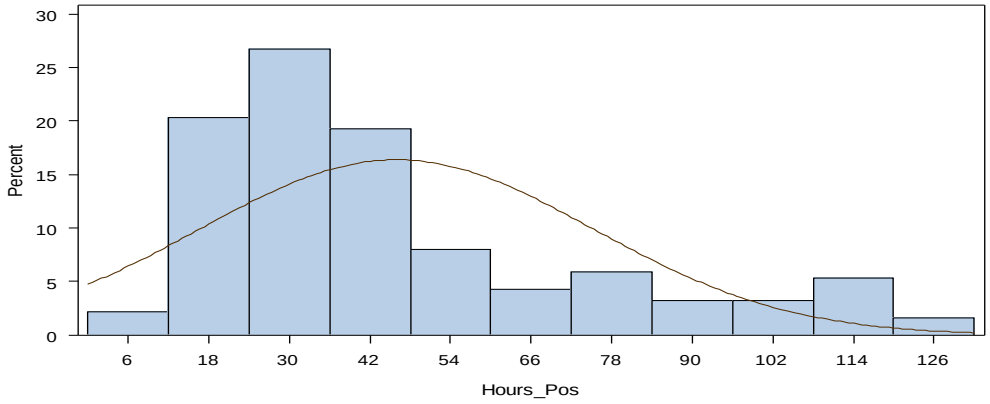


Table 1: Species' culture and characteristic frequencies for positive *Candida* blood cultures at Emory Healthcare associated facilities in 2009.

Species	No. of Cx (%)	No. w/pseudo-hyphae (%)	No. w/o pseudo-hyphae (%)	Mean hrs. to positive (SD)	No. aerobic (%)	No. anerobic (%)
Albicans	139 (39.9%)	54 (38.8%)	85 (61.2%)	42.6 (27.2)	87 (62.6%)	43 (30.9%)
Glabrata	118 (33.9%)	0 (0.0%)	118 (100%)	57.6 (35.8)	47 (39.8%)	70 (59.3%)
Guillermondi	6 (1.7%)	0(0.0%)	6 (100%)	21.4 (6.3)	2 (33.3%)	3 (50%)
Krusei	9 (2.6%)	1 (11.1%)	8 (88.9%)	32.9 (5.0)	2 (22.2%)	7 (77.8%)
Lusitaniae	4 (1.1%)	0 (0.0%)	4 (100%)	23.1 (5.3)	3 (75%)	1 (25%)
Parapsilosis	41 (11.8%)	3 (7.3%)	38 (92.7%)	46.3 (24.4)	37 (90.2%)	4 (9.8%)
Pararugosa	1 (0.3%)	0 (0.0%)	1 (100%)	71.9	1 (100%)	0 (0.0%)
Tropicalis	29 (8.3%)	7 (24.1%)	22 (75.9%)	31.5 (11.9)	14 (48.3%)	15 (51.7%)
Unknown*	1 (0.3%)	0 (0.0%)	1 (100%)	64.2	1 (100%)	0 (0.0%)
Total	348 (100%)	65 (18.7%)	283 (81.3%)	45.9 (29.2)	194 (55.7%)	143 (41.1%)

*Species never confirmed

Table 2: Facility specific species frequencies of positive *Candida* blood cultures at Emory Healthcare associated facilities in 2009.

Facility	Mean Hrs to positive (SD)	Albicans (%*)	Glabrata (%*)	Parapsilosis (%*)	Tropicalis (%*)	Other (%*)	Total (%^)
Emory University Hospital	43.4 (27.2)	104 (53.9%)	44 (22.8%)	11 (5.7%)	15 (7.8%)	19 (9.8%)	193 (55.5%)
Emory Midtown Hospital	53.6 (32.0)	32 (28.3%)	52 (46.0%)	18 (15.9%)	10 (8.8%)	1 (0.9%)	113 (32.5%)
Other	37.3 (27.4)	3 (7.1%)	22 (52.4%)	12 (28.6%)	4 (9.5%)	1 (2.4%)	42 (12.1%)
Total		139	118	41	29	21	348

*% of facility total, ^% of total cultures

No *C. glabrata* specimens were noted to have pseudohyphae, thus the presence of pseudohyphae had a negative predictive value of 100% (94.4, 100). Positive predictive value of the absence of pseudohyphae was 41.7% (36.1, 47.5). Sensitivity was 100% (96.9, 100) and specificity was 28.26% (22.8, 34.4).

Univariate analysis of hours to positive, aerobic/anaerobic growth, facility, and shift the culture became positive showed only hours to positive ($p<.001$) and aerobic/anaerobic ($p< .002$) growth status to be significant for identification of *C. glabrata.* Preliminary regression models included hours to positive, aerobic status, and facility. There was a significant difference ($p<.001$) in the hours to positive noted for *C. glabrata* cultures that grew in anerobic bottles (n=71, 60%); *C. glabrata* specimens that grew in anerobic bottles had a mean hours to positive of 46.7 (SD=18.1) hours whereas those that grew in aerobic bottles only had a mean hours to positive of 76.1 (SD=25.1) hours. Facility was included in initial models due to the possible confounding effect of the difference in hours to positive per facility. Backward elimination was

used to assess for interaction and confounding and produce a final regression model. No significant interaction was detected in the model that used aerobic status as the primary exposure variable; time to positive was noted to be a confounder and was left in the model. There was a significant interaction between hours to positive and aerobic status in the model using time to positive as the primary variable (Appendix B), but no confounding was noted.

Using the proc genmod procedure in SAS, the risk for *C. glabrata* in a yeast culture without pseudohyphae that grows in an anaerobic bottle, controlling for hours to positive and facility, was determined to be more than 3 times that of other *Candida* species (RR=3.00, CI 2.0, 4.6, $p < .0001$). Alternatively, the risk for *C. Glabrata* in a yeast culture without pseudohyphae that takes greater than 36 hours to become positive, controlling for aerobic status and facility, is many times that of other *Candida* species (RR = 11.40, CI 1.6, 83.0, $p = .016$).

Discussion

Candida glabrata is currently the second most common cause of *Candida* blood stream infections in the United States yet patients with *C. glabrata* infection are less likely to receive appropriate initial therapy than those patients with *Candida* infections caused by other species (Klevay et al. 2009). In addition to the financial burden of prolonged hospital stays, the loss of life is significant. A study by Klevay et al. (2008) concluded that patients receiving appropriate therapy for *C. glabrata* infection had lower mortality rates than those who did not (35% versus 71%). The research presented here offers a simple, cost effective method for potentially timelier identification of *C. glabrata* candidemia and earlier initiation of appropriate therapy.

Overuse of echinocandins, the class of antifungal medication that *C. glabrata* is susceptible to, is not a viable alternative. Not only are echinocandins more expensive to purchase and administer, increased exposure of *Candida* species to these drugs increases the risk of

developing even greater resistance. Though the FDA has broadened initiatives for antimicrobial development to decrease time to market, relatively few new antifungal medications are in the development pipeline.

In an attempt to identify patients who may be at risk for a *C. glabrata* bloodstream infection, many studies have been done to isolate patient risk factors. Though no new risk factors have been identified in recent years the number of patients living with currently known risk factors has increased. In an attempt to facilitate treatment, other research has focused on determining the species of a *Candida* specimen as quickly as possible

The findings of this research concur with other studies that found an overall longer time to positive for *C. glabrata* blood cultures (Fernandez et al. 2009) when type of culture bottle was not taken into consideration. This study did, however, note a significantly shorter time to positive culture for *C. glabrata* growing in anaerobic bottles versus aerobic bottles which is supported by the findings of other researchers (Foster et al. 2007, Hui et al. 2009, and Hovrath et al. 2007). These findings are reflected in the high risk ratio noted in the model with time to positive as the exposure variable, controlling for aerobic status.

No studies were found in the literature that used the presence or absence of pseudohyphae as a method of clinical identification of yeast specimens. Until the late 1990's *Glabrata* was not classified as a *Candida* species due to its inability to form psuedohyphae; it was classified as the genera *Torulopsis.* Research into how experienced and inexperienced observers identified the presence of pseudohyphae on specimens of various yeast species was undertaken by Odds et al. (1997) to address the controversy surrounding the reclassification of *Torulopsis.* None of the experienced observers in this study indicated a true or unequivocal finding of hypha or

pseudohypha on any of the 108 *Glabrata* specimens. Only 10 *Glabrata* specimens were noted to have equivocal pseudohypha formations by one of four experienced observers. It is interesting that the Odds et al. study often had equivocal pseudohyphae noted on *C.parapsilosis* cultures by both experienced and inexperienced observers. Of the *C. parapsilosis* specimens from the Emory Healthcare system only 3 of 41 were noted to have pseudohyphae growing, a seemingly unusual finding. It may be that Emory lab technicians were more likely to dismiss equivocal growth. *C. parapsilosis* was very unlikely to grow in an anaerobic bottle, however (n=4), allowing for the differentiation of this species from *C. glabrata.*

Candida glabrata has characteristics unique to the *Candida* genera; no formation of pseudohyphae, the propensity to grow in an anerobic culture bottle, and slow growth in an aerobic culture bottle. This study has attempted to use these characteristics to aid in the early identification of *C. glabrata* bloodstream infections. In addition to the use of innate characteristics that can be assessed at little or no additional cost the strengths of this study include an adequate sample size, inclusion of 9 species of *Candida*, and inclusion of cultures from more than one facility. Weaknesses of the study include its retrospective nature and potential information bias related to the assumption that no notation of presence or absence of pseudohyphae is equal to no pseudohyphae.

Moving forward from this research it is recommended that a formal training program for the microbiology lab technicians be instituted to ensure that all technicians are able to identify pseudohyphae and note its absence as well as presence on initial lab reports. Information regarding pseudohyphae and bottle type where growth occurred, whether anaerobic, aerobic, or both, should be included in lab reports that can be accessed by clinicians and in verbal reports to nurses. Clinicians and other care providers should be educated on the significance of these

findings. Findings from this research could be prospectively reevaluated after these outreach programs have been implemented.

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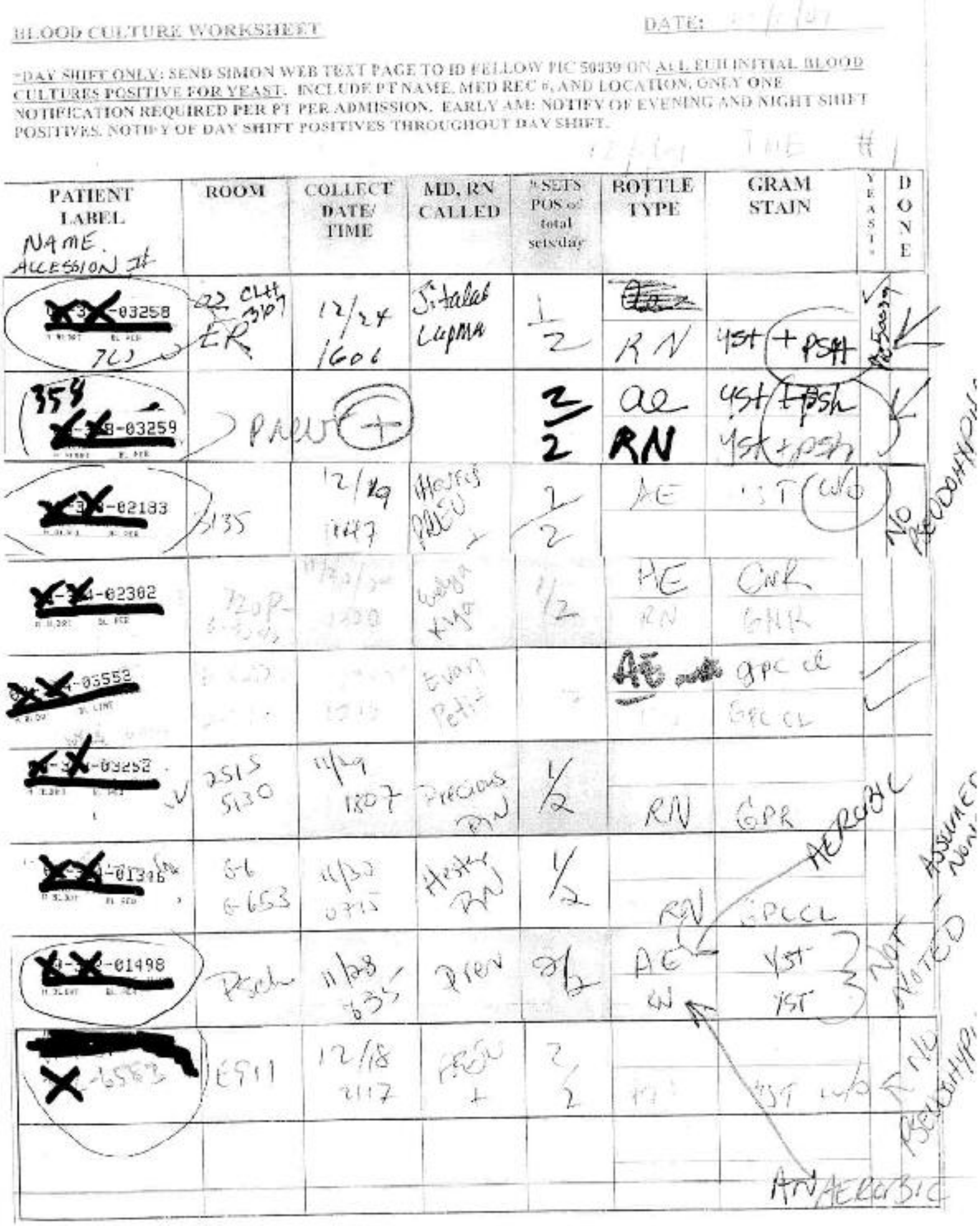
Appendix A: Microbiology worksheet


BLOOD CULTURE WORKSHEET

DATE:

*DAY SHIFT ONLY: SEND SIMON WEB TEXT PAGE TO ID FELLOW PIC 50339 ON ALL EUH INITIAL BLOOD CULTURES POSITIVE FOR YEAST. INCLUDE PT NAME, MED REC #, AND LOCATION. ONLY ONE NOTIFICATION REQUIRED PER PT PER ADMISSION. EARLY AM: NOTIFY OF EVENING AND NIGHT SHIFT POSITIVES. NOTIFY OF DAY SHIFT POSITIVES THROUGHOUT DAY SHIFT.

PATIENT LABEL NAME. ACCESSION #	ROOM	COLLECT DATE/ TIME	MD, RN CALLED	# SETS POS of total sets/day	BOTTLE TYPE	GRAM STAIN	YEAST	DONE
[redacted]-03258	ER	12/24 1606		1/2	RN	4st + PSH		
[redacted]-03259				2/2	ae RN	4st + psh		
[redacted]-02183	5135	12/29		1/2	AE			
[redacted]-02382				1/2	AE RN			
[redacted]-03552					AE	gpc cl		
[redacted]-03252	2515 5130	11/29 1807	RN	1/2	RN	GPR		
[redacted]-01316	6-653		RN	1/2	RN	GPCCL		
[redacted]-01498				2/2	AE	Yst		
[redacted]	E511	12/18 2117		2/2				

Appendix B:

2X2 Table of Pseudohyphae Presence used for analysis of PPV, NPV, Specificity and Sensitivity

	Candida glabrata	**Other *Candida***	**Total**	
No Pseudohyphae	118	165	283	PPV=41.7%
Pseudohyphae	0	65	65	NPV=100%
Total	118	230	348	
	Sensitivity = 100%	Specificity = 28.3%		

Positive Predictive Value: 118/283 = 41.7%

Negative Predictive Value: 65/65 = 100%

Sensitivity: 118/118 = 100%

Specificity: 65/230 = 28.3%

Appendix C: SAS output

The SAS System

The CONTENTS Procedure

Data Set Name	MYLIB.AVGHR	Observations	349
Member Type	DATA	Variables	9
Engine	V9	Indexes	0
Created	Thursday, May 06, 2010 08:56:00 PM	Observation Length	56
Last Modified	Thursday, May 06, 2010 08:56:00 PM	Deleted Observations	0
Protection		Compressed	NO
Data Set Type		Sorted	NO
Label			
Data Representation	WINDOWS_32		
Encoding	wlatin1 Western (Windows)		

Engine/Host Dependent Information

Data Set Page Size	8192
Number of Data Set Pages	3
First Data Page	1
Max Obs per Page	145
Obs in First Data Page	109
Number of Data Set Repairs	0
Filename	C:\Users\Gwen\Documents\My SAS Files\avghr.sas7bdat
Release Created	9.0201M0
Host Created	W32_VSHOME

Alphabetic List of Variables and Attributes

#	Variable	Type	Len	Format	Informat	Label
1	Accession_	Char	12	$12.	$12.	Accession
6	Aerobic	Num	8			Aerobic
9	Deceased	Char	3	$3.	$3.	Deceased
8	Facility	Num	8			Facility
5	Hours_Pos_	Num	8			Hours_Pos
4	MRN	Char	3	$3.	$3.	MRN
3	Pseudohyphae	Char	3	$3.	$3.	Pseudohyphae
7	Shift	Char	3	$3.	$3.	Shift
2	_Species	Num	8			Species

The SAS System

Full model with aerobic status as exposure variable

The GENMOD Procedure

Model Information

Data Set	WORK.AVERAGE	
Distribution	Poisson	
Link Function	Log	
Dependent Variable	_Species	Species

Number of Observations Read	283
Number of Observations Used	260
Missing Values	23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Aerobic
Prm3	Hours_Pos_
Prm4	Facility
Prm5	Aerobic*Hours_Pos_
Prm6	Aerobic*Facility

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	254	137.6041	0.5417
Scaled Deviance	254	137.6041	0.5417
Pearson Chi-Square	254	126.5305	0.4982
Scaled Pearson X2	254	126.5305	0.4982
Log Likelihood		-177.8020	
Full Log Likelihood		-177.8020	
AIC (smaller is better)		367.6041	
AICC (smaller is better)		367.9361	
BIC (smaller is better)		388.9682	

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-3.9067	0.5931	-5.0692	-2.7442	43.38	<.0001
Aerobic	1	2.4583	0.7247	1.0378	3.8787	11.51	0.0007
Hours_Pos_	1	0.0245	0.0048	0.0151	0.0340	25.97	<.0001

The GENMOD Procedure

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Facility	1	0.5689	0.2170	0.1436	0.9941	6.87	0.0088
Aerobic*Hours_Pos_	1	-0.0129	0.0081	-0.0287	0.0029	2.56	0.1099
Aerobic*Facility	1	-0.3153	0.2671	-0.8388	0.2081	1.39	0.2377

Scale	0	1.0000	0.0000	1.0000	1.0000

NOTE: The scale parameter was held fixed.

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits	
aerobic	11.6848	2.8231	48.3632	2.4583	0.7247	0.05	1.0378	3.8787
Exp(aerobic)				11.6848	8.4684	0.05	2.8231	48.3632

Contrast Estimate Results

Label	Chi-Square	Pr > ChiSq
aerobic	11.51	0.0007
Exp(aerobic)		

The SAS System

Removing aerobic*facility interaction variable

The GENMOD Procedure

Model Information

Data Set	WORK.AVERAGE
Distribution	Poisson
Link Function	Log
Dependent Variable	_Species Species

Number of Observations Read	283
Number of Observations Used	260
Missing Values	23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Aerobic
Prm3	Hours_Pos_
Prm4	Facility
Prm5	Aerobic*Hours_Pos_

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	255	139.0013	0.5451
Scaled Deviance	255	139.0013	0.5451
Pearson Chi-Square	255	126.1680	0.4948
Scaled Pearson X2	255	126.1680	0.4948

Log Likelihood	-178.5007
Full Log Likelihood	-178.5007
AIC (smaller is better)	367.0013
AICC (smaller is better)	367.2375
BIC (smaller is better)	384.8047

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-3.4974	0.4642	-4.4071	-2.5877	56.78	<.0001
Aerobic	1	1.8681	0.5161	0.8565	2.8796	13.10	0.0003
Hours_Pos_	1	0.0244	0.0048	0.0150	0.0339	25.68	<.0001
Facility	1	0.3587	0.1252	0.1133	0.6042	8.20	0.0042

The GENMOD Procedure

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Aerobic*Hours_Pos_	1	-0.0129	0.0081	-0.0287	0.0030	2.54	0.1110
Scale	0	1.0000	0.0000	1.0000	1.0000		

NOTE: The scale parameter was held fixed.

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits	
aerobic	6.4757	2.3549	17.8073	1.8681	0.5161	0.05	0.8565	2.8796
Exp(aerobic)				6.4757	3.3422	0.05	2.3549	17.8073

Contrast Estimate Results

Label	Chi-Square	Pr > ChiSq
aerobic	13.10	0.0003
Exp(aerobic)		

Removing aerobic*Hours_pos_ interaction variable and facility variable to check for confounding

The GENMOD Procedure

Model Information

Data Set	WORK.AVERAGE

Distribution	Poisson
Link Function	Log
Dependent Variable	_Species Species

Number of Observations Read	283
Number of Observations Used	260
Missing Values	23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Aerobic
Prm3	Hours_Pos_

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	257	149.2713	0.5808
Scaled Deviance	257	149.2713	0.5808
Pearson Chi-Square	257	133.7104	0.5203
Scaled Pearson X2	257	133.7104	0.5203
Log Likelihood		-183.6357	
Full Log Likelihood		-183.6357	
AIC (smaller is better)		373.2713	
AICC (smaller is better)		373.3651	
BIC (smaller is better)		383.9534	

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-2.5268	0.3183	-3.1506	-1.9030	63.03	<.0001
Aerobic	1	1.0992	0.2167	0.6746	1.5239	25.74	<.0001
Hours_Pos_	1	0.0199	0.0039	0.0123	0.0274	26.37	<.0001
Scale	0	1.0000	0.0000	1.0000	1.0000		

NOTE: The scale parameter was held fixed.

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits	
aerobic	3.0019	1.9632	4.5902	1.0992	0.2167	0.05	0.6746	1.5239

Exp(aerobic)	3.0019	0.6504	0.05	1.9632	4.5902

Contrast Estimate Results

Label	Chi-Square	Pr > ChiSq
aerobic	25.74	<.0001
Exp(aerobic)		

The GENMOD Procedure

Model Information

Data Set	WORK.AVERAGE
Distribution	Poisson
Link Function	Log
Dependent Variable	_Species Species

Number of Observations Read	283
Number of Observations Used	260
Missing Values	23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Aerobic

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	258	173.2591	0.6715
Scaled Deviance	258	173.2591	0.6715
Pearson Chi-Square	258	151.0000	0.5853
Scaled Pearson X2	258	151.0000	0.5853
Log Likelihood		-195.6295	
Full Log Likelihood		-195.6295	
AIC (smaller is better)		395.2591	
AICC (smaller is better)		395.3058	
BIC (smaller is better)		402.3805	

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq

Intercept	1	-1.2993	0.1601	-1.6131	-0.9854	65.84	<.0001
Aerobic	1	0.7856	0.1998	0.3940	1.1772	15.46	<.0001
Scale	0	1.0000	0.0000	1.0000	1.0000		

NOTE: The scale parameter was held fixed.

The SAS System

Less than 10% difference: facility is not a confounder and was left out of model

The model below removes hour to positive variable. It demonstrates hours to positive is a confounder.

The GENMOD Procedure

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits	
aerobic	2.1937	1.4829	3.2454	0.7856	0.1998	0.05	0.3940	1.1772
Exp(aerobic)				2.1937	0.4383	0.05	1.4829	3.2454

Contrast Estimate Results

Label	Chi-Square	Pr > ChiSq
aerobic	15.46	<.0001
Exp(aerobic)		

The SAS System

Final model

The GENMOD Procedure

Model Information

Data Set	WORK.AVERAGE
Distribution	Poisson
Link Function	Log
Dependent Variable	_Species Species

Number of Observations Read	283
Number of Observations Used	260
Missing Values	23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Aerobic
Prm3	Hours_Pos_

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	257	149.2713	0.5808
Scaled Deviance	257	149.2713	0.5808
Pearson Chi-Square	257	133.7104	0.5203
Scaled Pearson X2	257	133.7104	0.5203
Log Likelihood		-183.6357	
Full Log Likelihood		-183.6357	
AIC (smaller is better)		373.2713	
AICC (smaller is better)		373.3651	
BIC (smaller is better)		383.9534	

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-2.5268	0.3183	-3.1506	-1.9030	63.03	<.0001
Aerobic	1	1.0992	0.2167	0.6746	1.5239	25.74	<.0001
Hours_Pos_	1	0.0199	0.0039	0.0123	0.0274	26.37	<.0001
Scale	0	1.0000	0.0000	1.0000	1.0000		

NOTE: The scale parameter was held fixed.

The SAS System

Final model

The SAS System

The CONTENTS Procedure

Data Set Name	MYLIB.HILOW36	Observations	348
Member Type	DATA	Variables	9
Engine	V9	Indexes	0
Created	Thursday, May 06, 2010 10:22:53 PM	Observation Length	56
Last Modified	Thursday, May 06, 2010 10:22:53 PM	Deleted Observations	0
Protection		Compressed	NO
Data Set Type		Sorted	NO
Label			
Data Representation	WINDOWS_32		
Encoding	wlatin1 Western (Windows)		

Engine/Host Dependent Information

Data Set Page Size	8192
Number of Data Set Pages	3
First Data Page	1
Max Obs per Page	145
Obs in First Data Page	109
Number of Data Set Repairs	0
Filename	C:\Users\Gwen\Documents\My SAS Files\hilow36.sas7bdat

Release Created 9.0201M0
Host Created W32_VSHOME

Alphabetic List of Variables and Attributes

#	Variable	Type	Len	Format	Informat	Label
1	Accession_	Char	12	$12.	$12.	Accession
6	Aerobic	Num	8			Aerobic
9	Deceased	Char	3	$3.	$3.	Deceased
8	Facility	Num	8			Facility
5	Hours_Pos_	Num	8			Hours_Pos
4	MRN	Char	3	$3.	$3.	MRN
3	Pseudohyphae	Char	3	$3.	$3.	Pseudohyphae
7	Shift	Char	3	$3.	$3.	Shift
2	Species	Num	8			Species

The SAS System

Model with hours to positive as exposure variable

The GENMOD Procedure

Model Information

Data Set	WORK.OVER36
Distribution	Poisson
Link Function	Log
Dependent Variable	Species Species

Number of Observations Read	283
Number of Observations Used	260
Missing Values	23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Hours_Pos_
Prm3	Aerobic
Prm4	Facility
Prm5	Hours_Pos_*Aerobic
Prm6	Hours_Pos_*Facility

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	254	148.2102	0.5835
Scaled Deviance	254	148.2102	0.5835
Pearson Chi-Square	254	153.4671	0.6042
Scaled Pearson X2	254	153.4671	0.6042
Log Likelihood		-184.1051	
Full Log Likelihood		-184.1051	
AIC (smaller is better)		380.2102	

AICC (smaller is better)	380.5422
BIC (smaller is better)	401.5742

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-3.9602	1.0775	-6.0721	-1.8483	13.51	0.0002
Hours_Pos_	1	2.1513	1.1255	-0.0546	4.3571	3.65	0.0559
Aerobic	1	2.7947	1.0216	0.7924	4.7969	7.48	0.0062

The GENMOD Procedure

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Facility	1	0.2799	0.2304	-0.1717	0.7315	1.48	0.2244
Hours_Pos_*Aerobic	1	-2.0572	1.0447	-4.1047	-0.0096	3.88	0.0489
Hours_Pos_*Facility	1	0.1328	0.2732	-0.4026	0.6683	0.24	0.6269
Scale	0	1.0000	0.0000	1.0000	1.0000		

NOTE: The scale parameter was held fixed.

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits	
Hours_pos	8.5957	0.9469	78.0325	2.1513	1.1255	0.05	-0.0546	4.3571
Exp(Hours_pos)				8.5957	9.6741	0.05	0.9469	78.0325

Contrast Estimate Results

Label	Chi-Square	Pr > ChiSq
Hours_pos	3.65	0.0559
Exp(Hours_pos)		

Removed hours__pos_*facility

The GENMOD Procedure

Model Information

Data Set	WORK.OVER36
Distribution	Poisson
Link Function	Log

Dependent Variable Species Species

Number of Observations Read 283
Number of Observations Used 260
Missing Values 23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Hours_Pos_
Prm3	Aerobic
Prm4	Facility
Prm5	Hours_Pos_*Aerobic

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	255	148.4481	0.5821
Scaled Deviance	255	148.4481	0.5821
Pearson Chi-Square	255	156.4568	0.6136
Scaled Pearson X2	255	156.4568	0.6136
Log Likelihood		-184.2240	
Full Log Likelihood		-184.2240	
AIC (smaller is better)		378.4481	
AICC (smaller is better)		378.6843	
BIC (smaller is better)		396.2515	

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-4.1267	1.0245	-6.1347	-2.1186	16.22	<.0001
Hours_Pos_	1	2.3919	1.0131	0.4062	4.3776	5.57	0.0182
Aerobic	1	2.7891	1.0215	0.7869	4.7912	7.45	0.0063
Facility	1	0.3739	0.1237	0.1315	0.6162	9.14	0.0025

The GENMOD Procedure

Analysis Of Maximum Likelihood Parameter Estimates

Hours to positive has significant interaction with aerobic status

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Hours_Pos_*Aerobic	1	-2.0580	1.0447	-4.1057	-0.0104	3.88	0.0488

Scale	0	1.0000	0.0000	1.0000	1.0000

NOTE: The scale parameter was held fixed.

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits	
Hours_pos	10.9339	1.5010	79.6455	2.3919	1.0131	0.05	0.4062	4.3776
Exp(Hours_pos)				10.9339	11.0776	0.05	1.5010	79.6455

Contrast Estimate Results

Label	Chi-Square	Pr > ChiSq
Hours_pos	5.57	0.0182
Exp(Hours_pos)		

The SAS System

The GENMOD Procedure

Final model

Model Information

Data Set	WORK.OVER36
Distribution	Poisson
Link Function	Log
Dependent Variable	Species Species

Number of Observations Read	283
Number of Observations Used	260
Missing Values	23

Parameter Information

Parameter	Effect
Prm1	Intercept
Prm2	Hours_Pos_
Prm3	Aerobic
Prm4	Hours_Pos_*Aerobic

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	256	157.2397	0.6142
Scaled Deviance	256	157.2397	0.6142
Pearson Chi-Square	256	149.9999	0.5859
Scaled Pearson X2	256	149.9999	0.5859
Log Likelihood		-188.6199	

Full Log Likelihood	-188.6199
AIC (smaller is better)	385.2397
AICC (smaller is better)	385.3966
BIC (smaller is better)	399.4824

Algorithm converged.

Analysis Of Maximum Likelihood Parameter Estimates

Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-3.4965	1.0000	-5.4565	-1.5365	12.23	0.0005
Hours_Pos_	1	2.4336	1.0131	0.4480	4.4192	5.77	0.0163
Aerobic	1	2.8034	1.0215	0.8012	4.8055	7.53	0.0061
Hours_Pos_*Aerobic	1	-2.1319	1.0443	-4.1788	-0.0851	4.17	0.0412
Scale	0	1.0000	0.0000	1.0000	1.0000		

The GENMOD Procedure

NOTE: The scale parameter was held fixed.

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits	
Hours_pos	11.4000	1.5652	83.0293	2.4336	1.0131	0.05	0.4480	4.4192
Exp(Hours_pos)				11.4000	11.5490	0.05	1.5652	83.0293

Contrast Estimate Results

Label	Chi-Square	Pr > ChiSq
Hours_pos	5.77	0.0163
Exp(Hours_pos)		