PROFICIENCY TESTING SERVICE
AMERICAN ASSOCIATION OF BIOANALYSTS
205 West Levee St. ◆ Brownsville, TX 78520-5596
800-234-5315 ◆ 281-436-5357 ◆ Fax 713-781-5008

PARTICIPANT STATISTICS **THIRD QUADRIMESTER 2018**

GRAM STAIN

	Specimen 1		Specimen 2		Specimen 3		Specimen 4		Specimen 5		
Gram Stain	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**No. of Labs**
	68	5	1	72	10	63	1	72	70	3	73
Flagging		***	***		***		***			***	

	Specimen 1		Specimen 2		Specimen 3		Specimen 4		Specimen 5	
Morphology	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**
Cocci	20		64		58		3	***	0	***
Diplococci	37		1	***	3	***	2	***	0	***
No diplococci present	1	***	0		1		0		1	
Coccobacilli	4	***	0	***	0	***	16		0	***
Rods (Bacilli)	2	***	0	***	0	***	43		64	
Streptococci	1	***	0	***	3		1	***	0	***
Yeast	0	***	0	***	0	***	0	***	0	***
Total Population	**65**		**65**		**65**		**65**		**65**	

Educational

	Specimen 1				Specimen 2			
Gram Stain	**Neg**	**Pos**	**Yeast**	**No pathogen found**	**Neg**	**Pos**	**Yeast**	**No pathogen found**
	33	0	0	0	0	33	0	0

	Specimen 1		Specimen 2	
Morphology	**No.**		**No.**	
Cocci	0		9	
Diplococci	0		0	
No diplococci present	0		0	
Coccobacilli	0		0	
Rods (Bacilli)	32		0	
Streptococci	0		23	
Yeast	0		0	
Total Population	**32**		**32**	

Gram Stain #1 – Blood Culture (*Morganella morganii*)

Clinical Presentation:

A 65-year old man presented to the ED with complaints of low back pain, painful urination, fever, and chills that began earlier in the day. He underwent a lithotripsy to remove a urethral stone 1 week earlier and had a medical history significant for diabetes mellitus. On initial presentation, the patient was awake, alert, and ambulatory; his vital signs were: temperature, 103.2° F (39.6° C); heart rate, 132 beats/minute; respiratory rate, 28 breaths/minute; and BP, 80/50 mm Hg.

His physical exam was significant for lethargy, dry mucous membranes, costovertebral angle tenderness, and mottling bilaterally up to his knees. His lab tests included a white blood cell count of 1,000/mm3, lactic acid of 4.3 mmol/L and positive urine and blood cultures. A gram-stained smear from the positive blood culture can be visualized in the attached photomicrograph.

Discussion narrative:

This patient's clinical presentation was consistent with sepsis, secondary to a urinary tract infection (UTI) caused by *Morganella morganii*. Sepsis is a life-threatening condition that has an associated mortality of up to 41.1%. The urinary tract is the second most common infection site, accounting for approximately 20% to 40% of all severe cases of sepsis in patients that of was diagnosed with urosepsis. Common causes of urosepsis include obstructive etiologies (e.g., urethral stones, tumors, urethral strictures, etc.) and patients with indwelling urethral catheters, ureteric stents, and nephrostomy tubes, for example, Also, patients with a weakened immune system, such as older adults and patients with chronic metabolic disorders (e.g., diabetes mellitus, chronic kidney disease), AIDS, chronic corticosteroid use, etc., are also at a higher risk to develop urosepsis. The clinical signs of UTIs include fever, nausea, vomiting, flank pain, costovertebral angle tenderness, dysuria, hematuria, urinary retention, and urinary frequency. Given the high incidence and severity of sepsis, early recognition and appropriate management of UTIs play a vital role in preventing the disease progression to urosepsis.

M. morganii is an unusual opportunistic pathogen that is clinically and often isolated as a cause of nosocomial infection in adults, specifically in UTIs or wound infections. The urinary tract is the major portal for *M. morganii* entry, followed by the hepatobiliary tract, skin and soft tissue, and blood. However, *M. morganii* has been recognized an increasingly important pathogen because of its virulence and increasing drug resistance, which has resulted in a high mortality rate in some infections. The disease spectrum associated with *M. morganii* infections are diverse and include pyelonephritis, septic shock, urinary tract infection, osteomyelitis, peritonitis, abscess, meningitis, sepsis, pericarditis, pneumonia, bacteremia, septic arthritis, endophthalmitis, pancreatitis, pyomyositis, ulcer, cellulitis, and wound infection. *M. morganii* is a facultative anaerobic rod-shaped Gram-negative enteric bacterium. It is a motile, non-lactose fermenting bacterium, which shares with the *Proteus* members the capacity for urease production and presence of phenylalanine deaminase. *M. morganii* is also negative for citrate utilization. While disease prevalence with this pathogen is not high, the organism can be found in all major phenotypic and proteomic identification databases (that accompany current semi-automated ID platforms) from the various vendors. Importantly, *M. morganii* has intrinsic resistance to oxacillin, ampicillin, amoxicillin, most of the first- and second-generation cephalosporins, macrolides, lincosamides, glycopeptides, fosfomycin and colistin; whereas, this pathogen is normally sensitive to aztreonam, aminoglycosides, antipseudomonal penicillins, third- and fourth-generation cephalosporins, carbapenems, quinolones, and trimethoprim/sulfamethoxazole.

Gram Stain #2 – Skin lesion (*Streptococcus pyogenes*)

Clinical Presentation:

A man in his late fifties presented to an Emergency Department with a red painful middle finger on the right hand. Eighteen hours before presentation, he was pruning trees in his back yard and incurred a small abrasion on his right hand. Initial examination revealed a red, swollen finger. Drainage from a lateral incision was sent for culture, and the patient was prescribed cephalexin and discharged.

Twenty-one hours later, he returned to the emergency department (ED) with fever, chills, and increasing pain from the palm to the axilla. The Gram stain from the wound can be visualized in the attached photomicrograph.

Discussion narrative:

Upon the patient's return to the ED, he was administered penicillin, clindamycin and aztreonam emergently and immediately taken to the operating room for incision and debridement where a diagnosis of necrotizing fasciitis was confirmed. Consistent with the Gram stain (gram-positive cocci in long chains, the wound culture grew Group A Streptococcus (GAS; or *Streptococcus pyogenes*). *S. pyogenes* is an important global human pathogen that causes a wide variety of acute infections, such as soft tissue infections and pharyngitis; severe life-threatening infections, such as streptococcal toxic shock syndrome; and devastating postinfectious sequelae, such as rheumatic fever and glomerulonephritis. Streptococcal pharyngitis is the most common GAS-associated infection, but other clinical manifestations include: 1) superficial skin and soft tissue infections (e.g., impetigo, erysipelas, and cellulitis); 2) severe life-threatening infections (e.g., scarlet fever, bacteremia, necrotizing fasciitis, myonecrosis, and streptococcal toxic shock syndrome); and 3) postinfectious sequelae (e.g., acute rheumatic fever and poststreptococcal glomerulonephritis). The only known reservoirs for *S. pyogenes* are the skin and mucous membranes of the human host. Necrotizing soft tissue infections (i.e., as seen in this patient case) include necrotizing forms of fasciitis, myositis, and cellulitis and can include involvement of the epidermis, dermis, subcutaneous tissue, fascia, and muscle. These infections are characterized clinically by fulminant tissue destruction, systemic signs of toxicity, and high mortality. Importantly (as highlighted above), accurate diagnosis and appropriate treatment must include early surgical intervention and antibiotic therapy.

Identifying Group A Streptococcus from clinical specimens involves screening blood agar plates for the presence of b-hemolytic colonies. The typical appearance of *Streptococcus pyogenes* colonies after 24 hours of incubation at 35-37°C is dome-shaped with a smooth or moist surface and clear margins. They display a white-greyish color and have a diameter of >0.5 mm, and are surrounded by a zone of b-hemolysis that is often 2-4 times as large as the colony diameter. Microscopically, GAS appears gram-positive cocci (GPC), arranged in chains (as given in the attached image). After the detection of b-hemolytic colonies displaying typical *S. pyogenes* morphology and Gram stain of GPC in chains, catalase^{neg} testing confirms that the isolates represent streptococci. Additionally, (manual) testing can then be applied for definite species identification. That is, the PYR test is a rapid colorimetric method that can be used to distinguish GAS from other b-hemolytic streptococci with a similar morphology which tests for the presence of the enzyme pyrrolidonyl aminopeptidase (i.e., positive in GAS). A second (manual) method that can also be used to confirm a presumptive identification of GAS is Lancefield grouping which is a serologic technique (i.e., latex agglutination) that identifies specific carbohydrates present on the bacterial cell wall of b-hemolytic streptococci (i.e., group A confirms GAS). Other methods utilized in the identification of GAS, in general are: 1) automated phenotypic testing platforms; 2) rapid antigen; 3) molecular amplification tests; and 4) MALDI-TOF mass spectrometry.