

Manual to Molecular to MALDI – How Do I Choose?

September 25, 2023

Kristen L. Smith, PhD, D(ABMM)

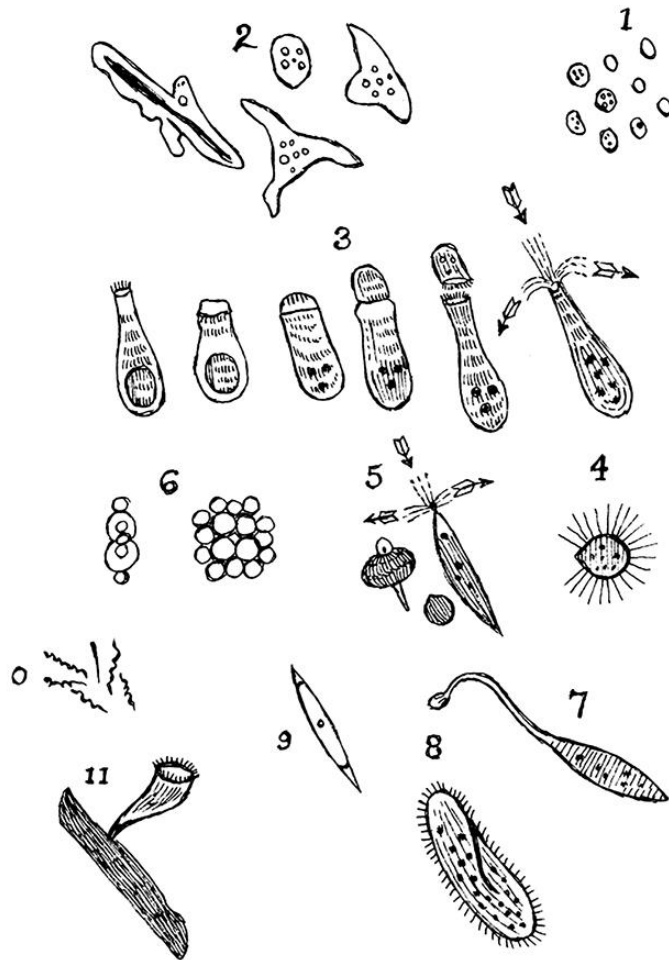
AGENDA

- “Micro” Microbiology History
- Manual Methods
- MALDI-TOF
- Brief Cases
- Care Team
- Discussion

“MICRO” MICROBIOLOGY HISTORY



BIRTH OF CLINICAL MICROBIOLOGY



LEEUWENHOEK'S
ANIMALCULES

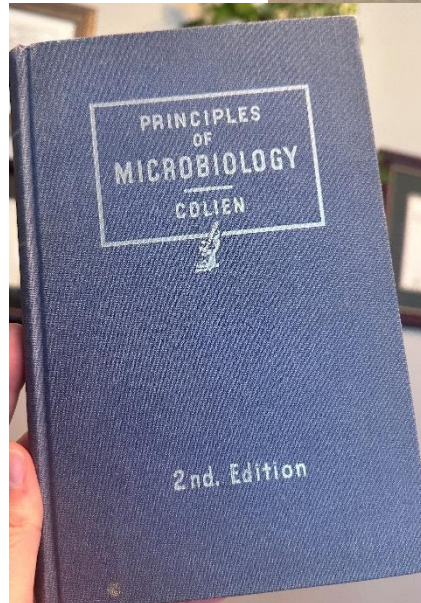
- “The Father” – Antonie van Leeuwenhoek
 - 1632-1723
- “The Master” – Louis Pasteur
 - 1822-1895
- “The Father” (Medical Microbiology) – Robert Koch
 - 1843 - 1910

CLINICAL MICRO TO THE 1950s

TABLE V
CHARACTERISTICS OF THE STREPTOCOCCI

GROUP	SPECIES	HEMOLYSIS	LANCZFELD GROUP	REDUCTION	NH ₄ FROM PEPTONE	SODIUM HIPPURATE	60° C. FOR 30 MIN.	MANNITOL	LACTOSE	TREHALOSE	SORBITOL	SALICIN	GROWTH AT		GROWTH IN PRESENCE OF	
													10° C.	45° C.		
Pyogenic	<i>S. pyogenes</i>	CZ	A	-	+	-	-	-	+	+	-	-	-	-	-	-
	<i>S. agalactiae</i>	CZ	B	-	+	+	-	-	+	+	-	-	-	-	-	-
	<i>S. equi</i>	CZ	C	-	+	-	-	-	+	+	-	-	-	-	-	-
	Group C "human"	CZ	C	-	+	-	-	-	+	+	-	-	-	-	-	-
Viridans	<i>S. salivarius</i>	G	-	-	-	-	+	-	+	+	+	+	+	+	+	+
	<i>S. equinus</i>	G	-	-	-	-	+	-	+	+	+	+	+	+	+	+
	<i>S. bovis</i>	G+	-	-	-	-	+	-	+	+	+	+	+	+	+	+
	<i>S. thermophilus</i>	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
Lactic	<i>S. lactis cremoris</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
	<i>S. faecalis</i>	G+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

CZ, Clear zone; G, green; +, variable.



Gram Stain (Hucker's Modification)

Ammonium oxalate crystal violet is prepared as follows:

Solution A:
 Crystal violet ----- 4.0 Gm.
 Alcohol, 95% ----- 20.0 c.c.

Solution B:
 Ammonium oxalate ----- 0.8 Gm.
 Distilled water ----- 80.0 c.c.

Mix solutions A and B and filter.

Lugol's Iodine Solution
 Iodine ----- 1.0 Gm.
 Potassium iodide ----- 2.0 Gm.
 Distilled water ----- 300.0 c.c.

Dissolve the iodine and potassium iodide in 25 c.c. of the water; heat gently if necessary. Make up to 300 c.c. with distilled water and filter.

Safranin for Counterstain
 Safranin (Y) ----- 1.0 Gm.
 Distilled water ----- 100.0 c.c.

Staining Procedure:

- Cover film on slide with crystal violet and allow to stand for 1 minute.
- Wash with water and cover with iodine solution for 1 minute.
- Pour off the iodine, wash with water, and decolorize with 95 percent alcohol for 30 seconds or until the washings are no longer blue.
- Cover with safranin and allow to stand for 1 minute.
- Wash with water, dry in air and examine.

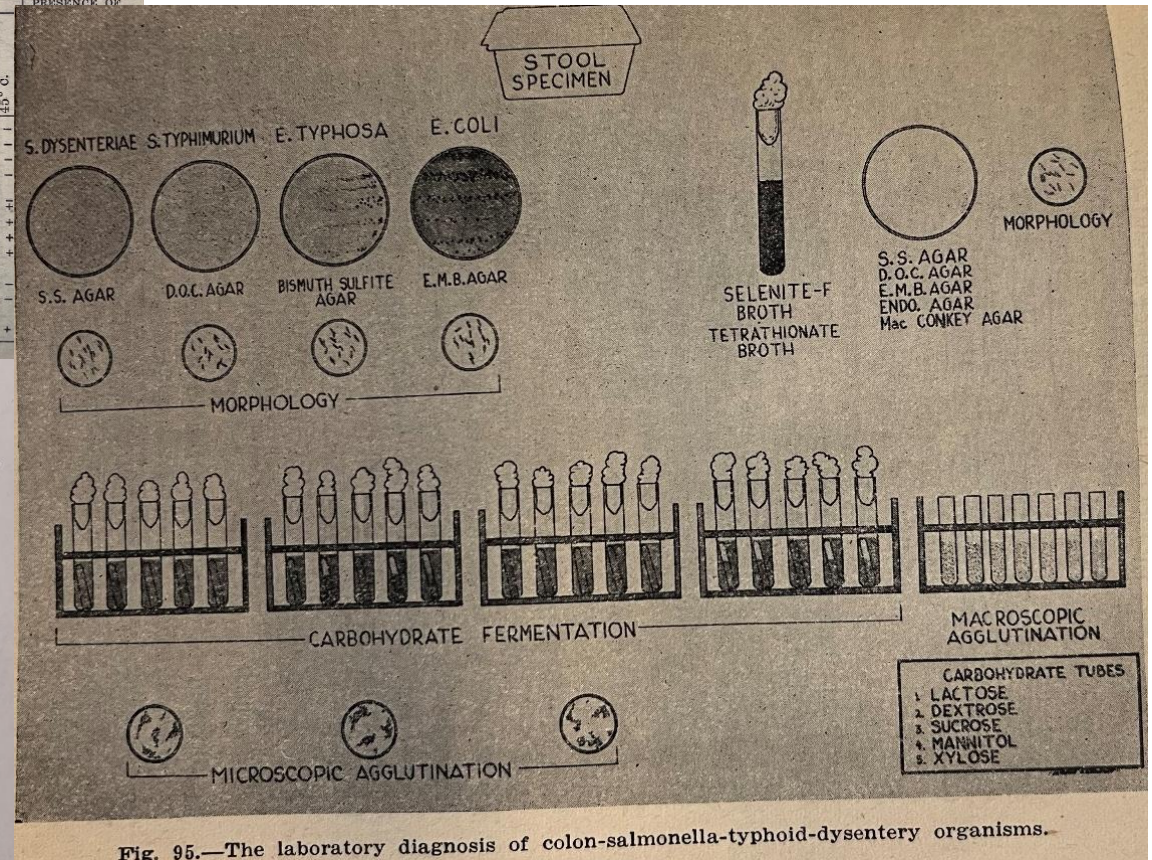


Fig. 95.—The laboratory diagnosis of colon-salmonella-typhoid-dysentery organisms.

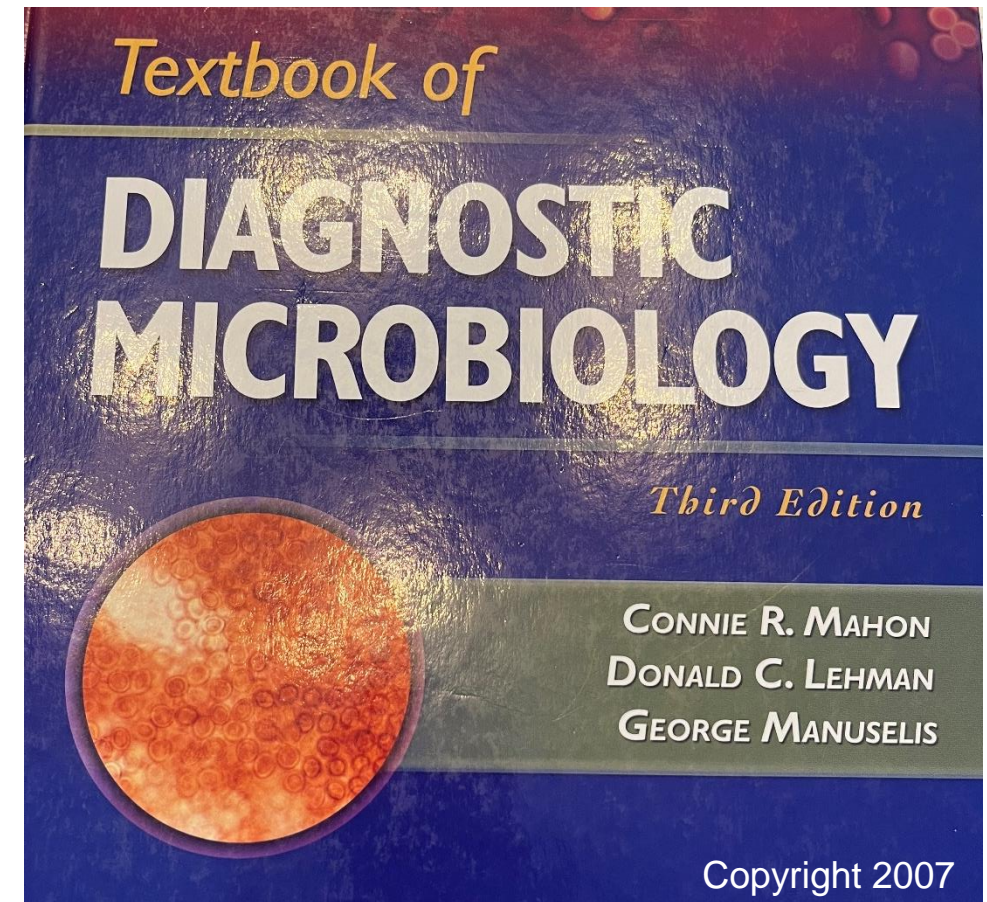
Mosby 1946

CLINICAL MICRO TO THE 1970s

Table 14-3 Differentiation among Staphylococci from Other Gram-Positive Cocci

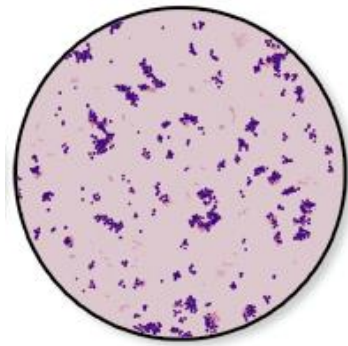
Characteristic	Staphylococci	Enterococci	Streptococci	Aerococci	Alloiococci	Planococci	Stomatococci	Macrococci	Micrococci	Rothia
Strict aerobe	-	-	-	-	-	-	-	-	-	-
Facultative anaerobe	d	+	+	+	+	+	+	±	+	-
Motility	-	d	-	-	-	-	-	-	-	+
Growth on NaCl agar										
5% NaCl	+	+	d	+	+	+	-	+	+	-
6.5% NaCl	+	+	d	+	+	+	-	+	+	-
12% NaCl	d	(±)	-	-	ND	+	-	+	+	-
Catalase	+	-	-	-	±	+	±	±	d	-
Benzidine test	+	-	-	-	±	+	±	+	+	±
Anaerobic acid from glucose	d	+	+	(+)	ND	-	+	+	-	+
Lysostaphin (200 µg/mL)	-	+	+	+	ND	+	+	-	±*	+
Erythromycin (0.04 µg/mL)	+	+	-	ND	ND	ND	ND	+	- [†]	ND
Bacitracin (0.04-U disk)	+	+	d	-	ND	ND	-	+	-	-

Modified from Bannerman TL: Staphylococcus, micrococcus, and other catalase: positive cocci that grow aerobically. In Murray PR et al, editors: *Manual of clinical microbiology*, ed 8, Washington, DC, 2003, ASM Press.
 +, 90% or more species or strains positive; ±, 90% or more species or strains weakly positive; -, 90% or more species or strains negative; d, 11% to 89% of species or strains positive; (), delayed reaction; ND, not determined.
 * Some strains of *M. luteus*, *M. roseus*, and *M. sedentarius* demonstrate susceptibility to lysostaphin, presumably because of contaminating levels of endo-β-N-acetylglucosaminidase activity.
 † A few *Micrococcus* strains demonstrate high-level (minimal inhibitory concentration >1 µg/mL) erythromycin resistance.



MANUAL METHODS

MANUAL METHODS

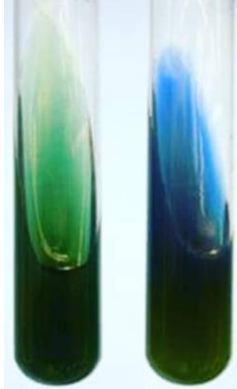


Medlineplus.gov

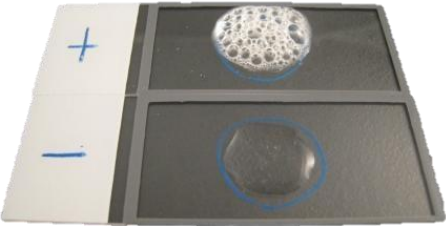


- Non-Selective
- Selective
- Solid vs. liquid
- Fungal
- AFB

MANUAL METHODS



Microbenotes.com



AUTOMATED IDENTIFICATIONS

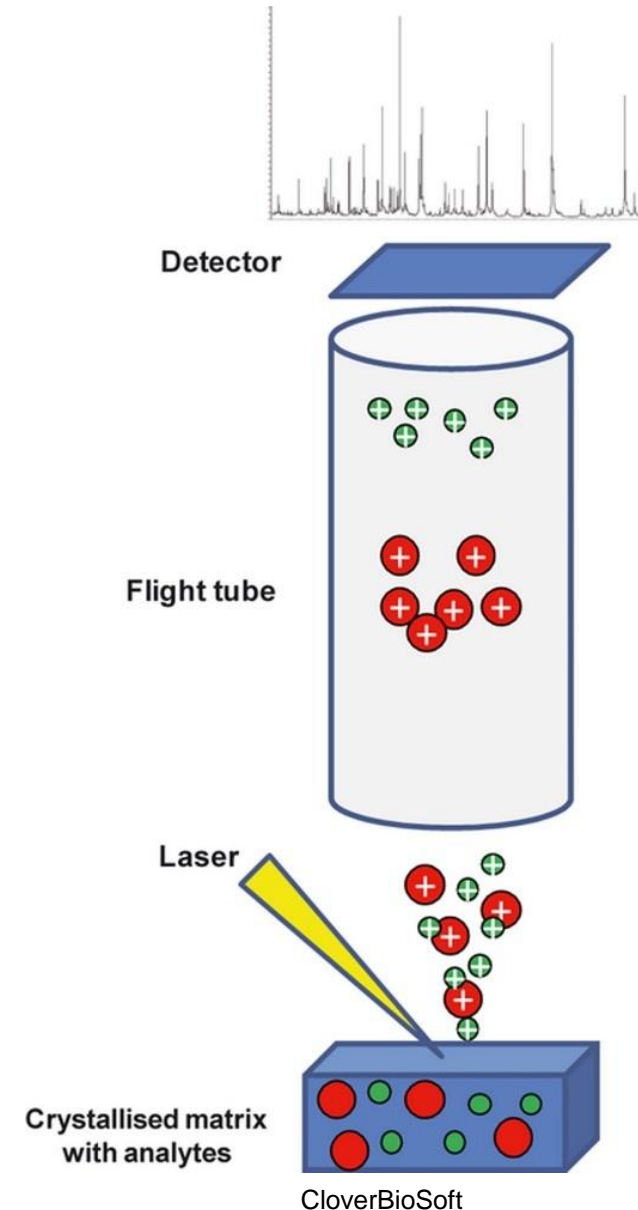
- Less hands on time
- Easier reagent management
- Space saving
- **Faster time to result**



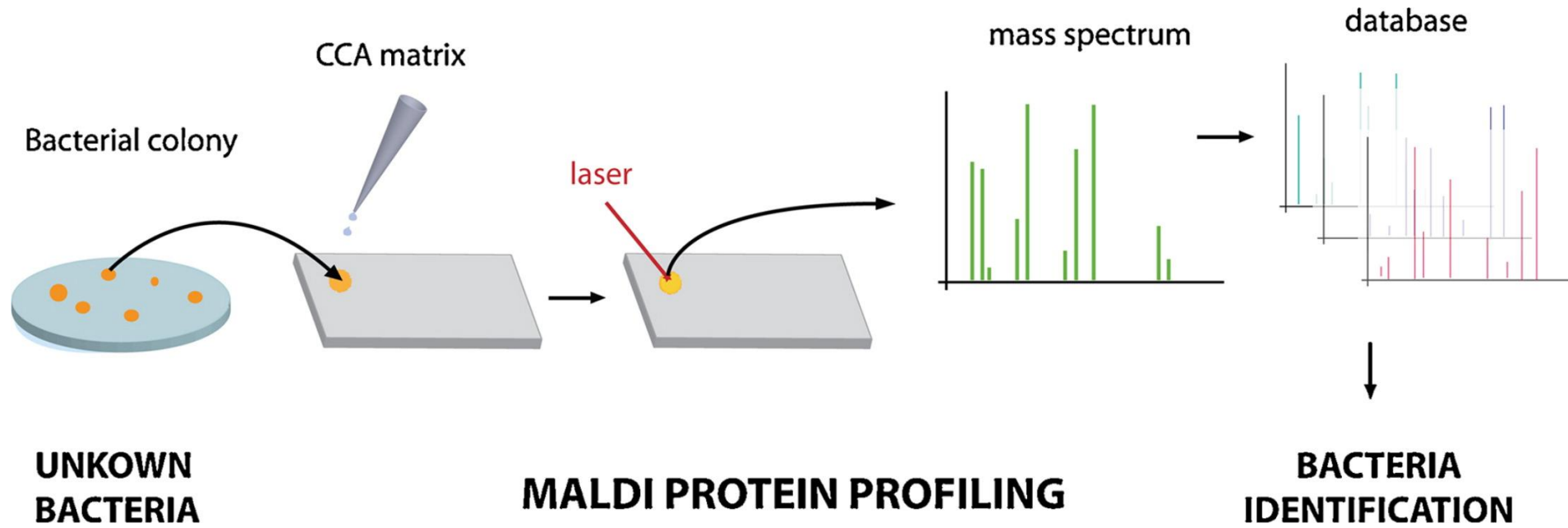
MALDI-TOF

MALDI-TOF

- Protein detection – MALDI-TOF
- Organism growth needed
- Organism + laser = protein fragments
- Can ID bacteria, yeasts, and molds
- Database dependent



MALDI-TOF



Identification of bacteria using mass spectrometry techniques. Krasney et al. 2013

MALDI-TOF

Direct From Specimen

Mycobacteria, Mold

Bacteria, Yeast



FUTURE MALDI?



Direct from Specimen



Resistance Profiles



Space Improvements



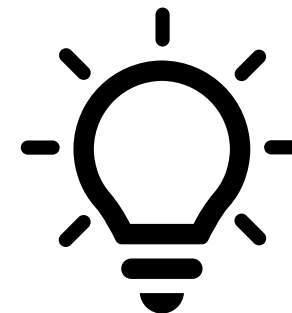
Fully Automated



Workflow Improvements



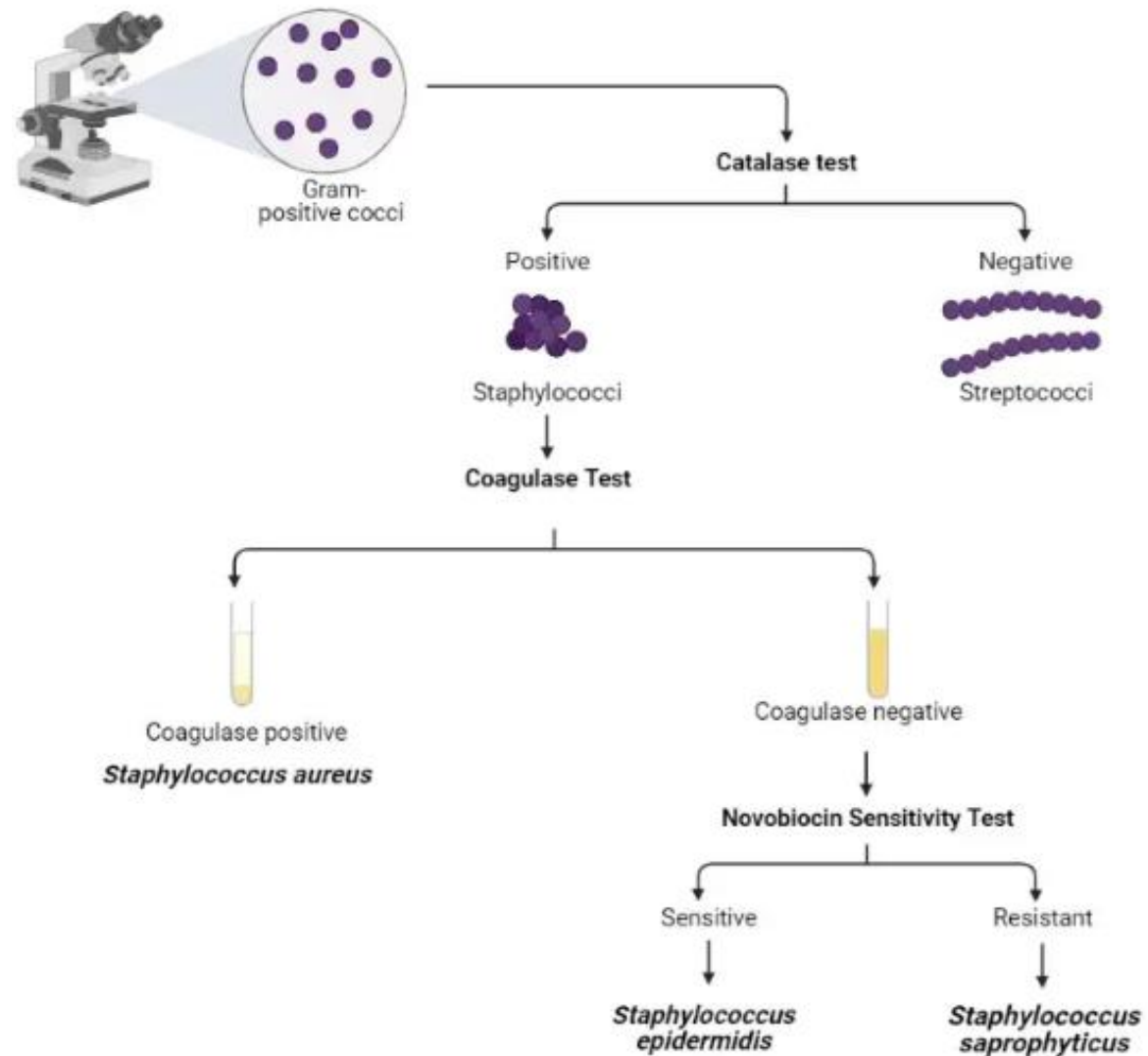
Affordability



Your thoughts?

BRIEF CASES

CASE STUDY 1



CASE STUDY 1

- Change in practice from CoNS to *Staphylococcus* XXX
- Realization that some *Staphylococcus* spp. can be pathogenic
- Limited information on antimicrobial susceptibility testing
 - Oxacillin resistance – *Staphylococcus haemolyticus* (87%), *Staphylococcus epidermidis* (71%), *Staphylococcus hominis* (54.1%)
- Increased the identification of CoNS by 85%

📄 | Research Article | 18 June 2015



Implementation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry in Routine Clinical Laboratories Improves Identification of Coagulase-Negative Staphylococci and Reveals the Pathogenic Role of *Staphylococcus lugdunensis*


Authors: [Xavier Argemi](#), [Philippe Riegel](#), [Thierry Lavigne](#), [Nicolas Lefebvre](#), [Nicolas Grandpré](#), [Yves Hansmann](#), [Benoit Jaulhac](#), [Gilles Prévost](#), [Frédéric Schramm](#) | [AUTHORS INFO & AFFILIATIONS](#)

CASE STUDY 2

- 71 year old male, end-stage lung disease presents for double lung transplant
- Pre- and Post-Transplant BALs exhibited yeast on Calcofluor white preparations
- 2 days post-transplant cultures were positive for yeast, no definitive ID by differential chromogenic media
- MALDI-TOF – no ID, VITEK2 Yeast ID – *Candida haemulonii*
- ID from CDC – *Candida auris*

JOURNAL ARTICLE

Donor-Derived Transmission of *Candida auris* During Lung Transplantation ^{FREE}

Marwan M Azar, Sarah E Turbett, Jay A Fishman, Virginia M Pierce 

Clinical Infectious Diseases, Volume 65, Issue 6, 15 September 2017, Pages 1040–1042,

<https://doi.org/10.1093/cid/cix460>

Published: 17 May 2017 [Article history](#) ▼

MOLECULAR DIAGNOSTICS



CARE TEAM



WHAT IS ANTIMICROBIAL STEWARDSHIP

- **“Stewardship”** – the activity of protecting and being responsible for something
- **“Antimicrobial stewardship”** involves practices to foster appropriate / prudent use of antimicrobials in human and animal health and plant protection ¹



1) WHO 2016

A PRUDENT, RESPONSIBLE USE OF ANTIBIOTICS

A set of interventions for improving antimicrobial prescribing:

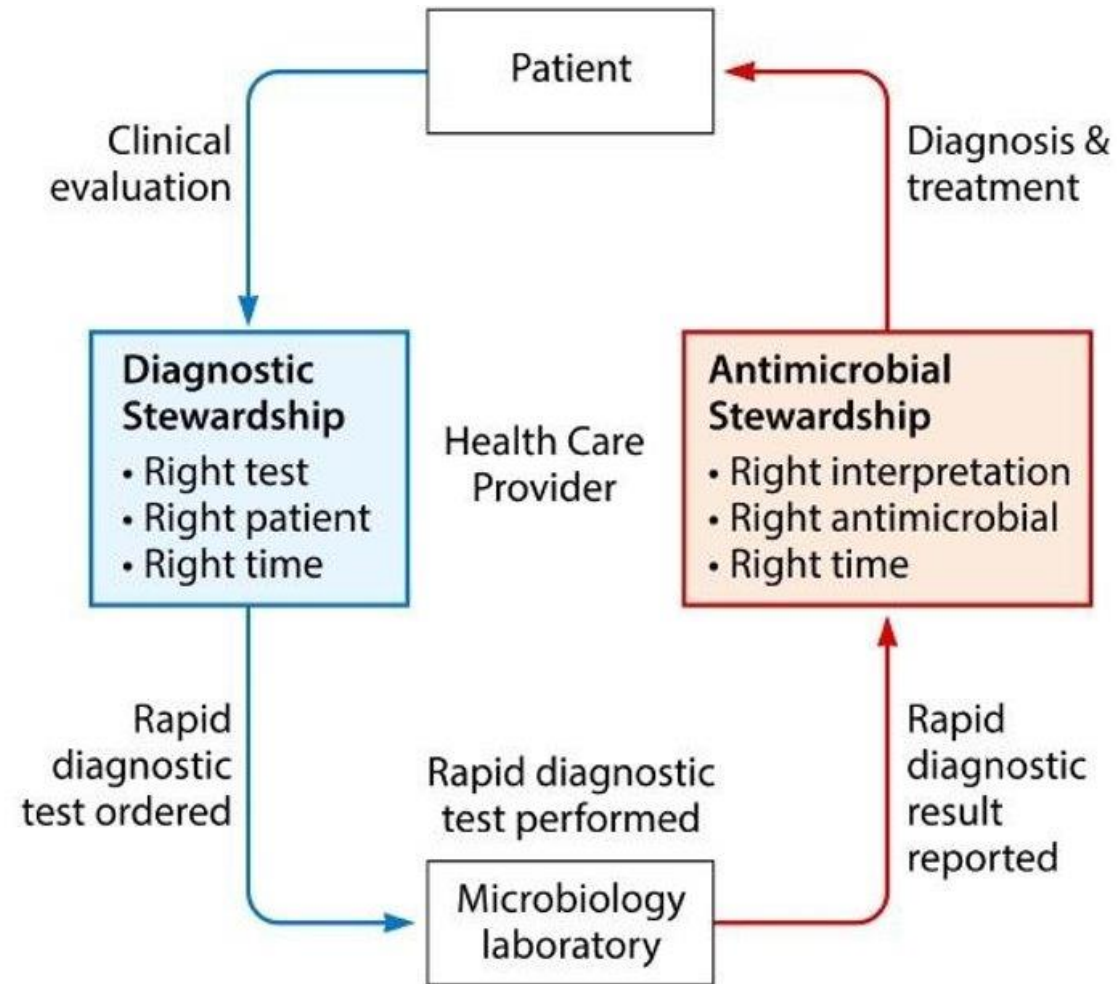
- Right antibiotic
- Right indication
- Right dose & route
- Right duration
- Causing the least harm to the patient and future patients

CDC



Diagnostic testing has a key-role to play !

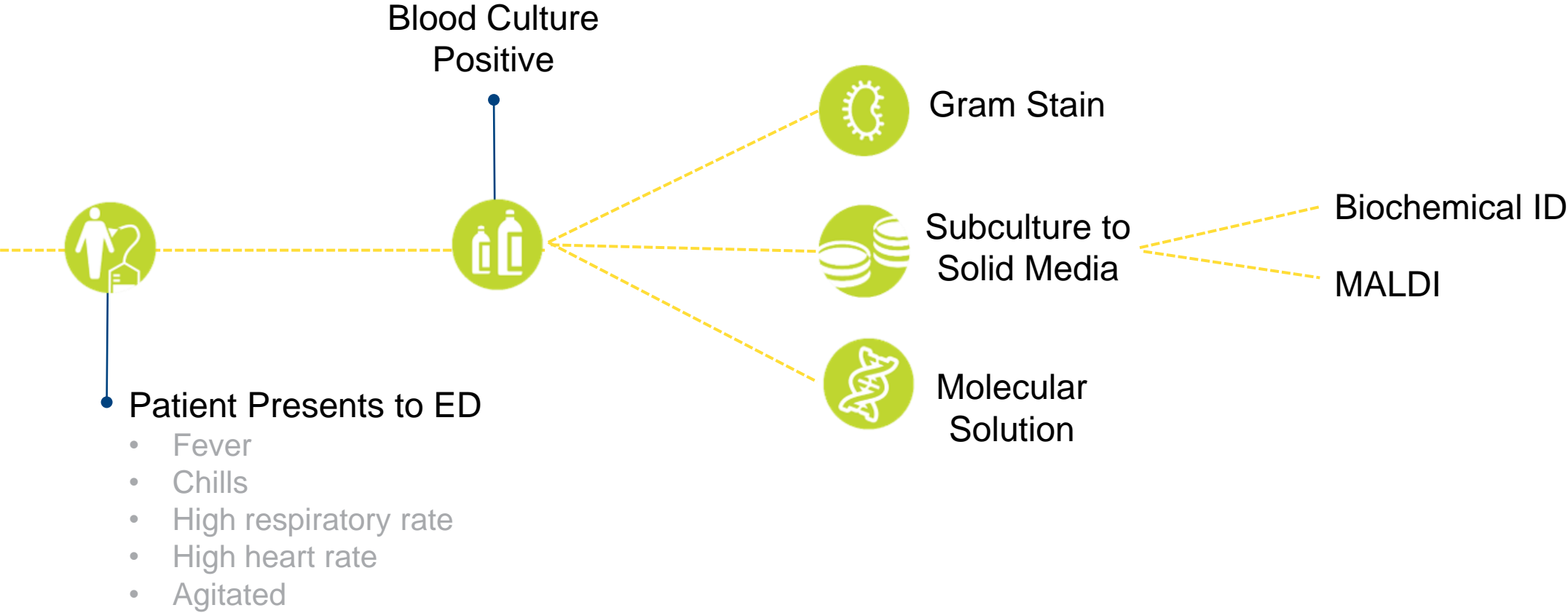
ANTIMICROBIAL STEWARDSHIP TEAM



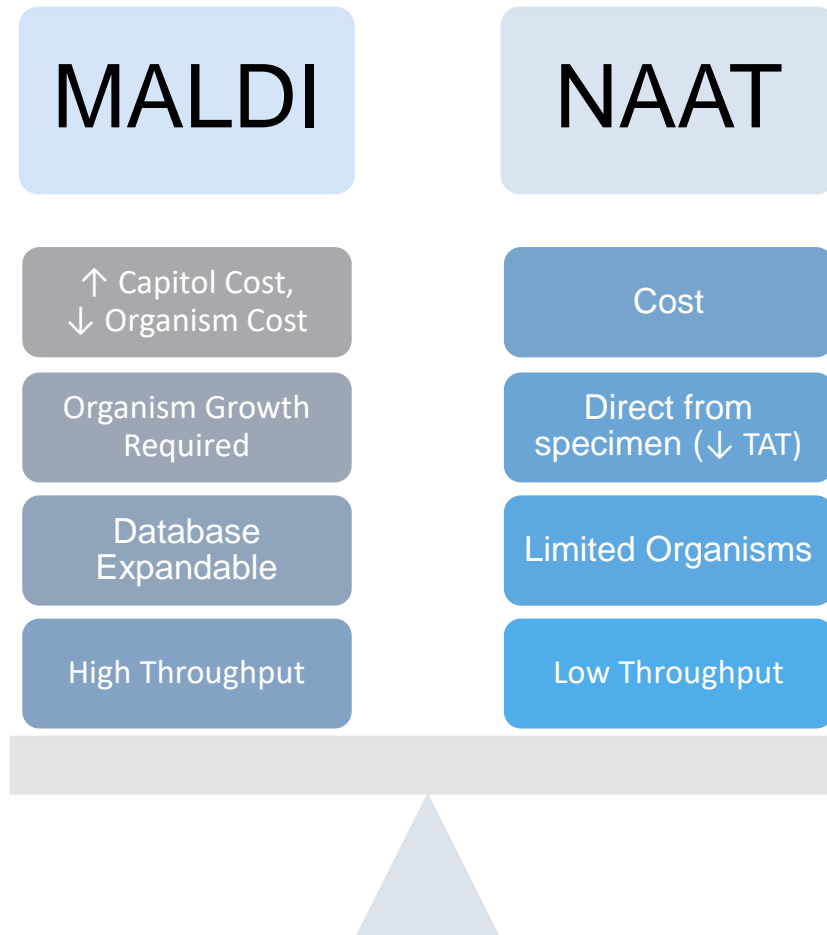
Messacar K, et al. J Clin Microbiol. 2016.

DISCUSSION

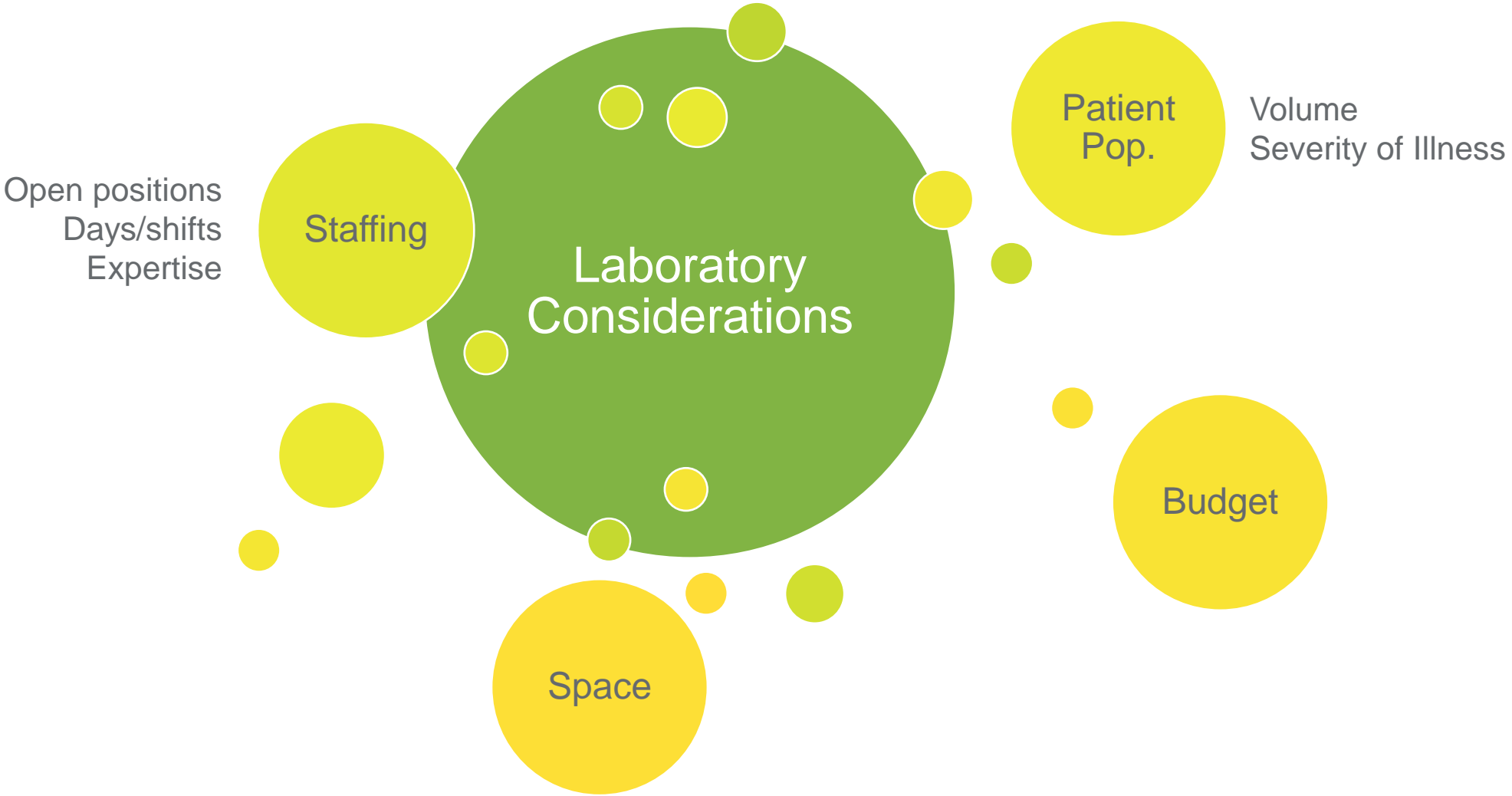
SEPSIS WORKFLOW



MALDI VS. NAAT?



WHAT DO YOU CHOOSE?





Kristen.Smith1@biomerieux.com

PIONEERING DIAGNOSTICS

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