SBAC
Sociedade Brasileira de Análises Clínicas

Metodologia de Coloração de Gram no Laboratório de Rotina

Alessandro C. O. Silveira

46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

Declaro não possuir conflitos de interesse.

SBAC
Sociedade Brasileira de Análises Clínicas

46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

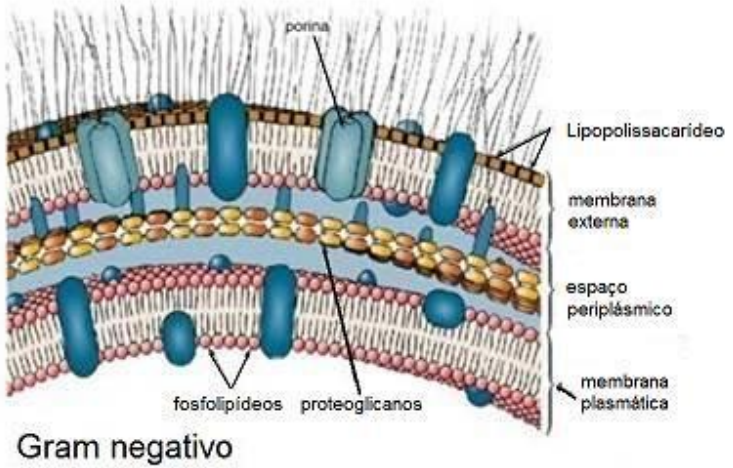
DINÂMICA

- ✓ Conceitos Gerais
- ✓ Indicações
- ✓ Padronização
- ✓ Morfologia
- ✓ Aplicações
- ✓ Automação
- ✓ Laudos
- ✓ Conclusões

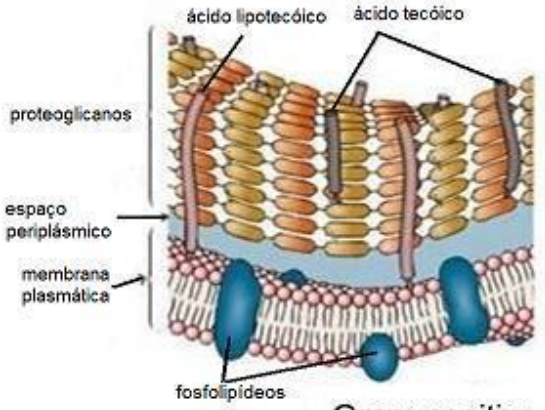
SBAC
Sociedade Brasileira de Análises Clínicas

CONCEITOS GERAIS

- ✓ Método simples, rápido e barato
- ✓ Avalia as propriedades morfo-tintoriais
- ✓ Excelente custo benefício
- ✓ Subjetividade
- ✓ Requer treinamento constante e padronização
- ✓ Necessidade de controle de qualidade diário
- ✓ Indispensável para uma microbiologia de qualidade

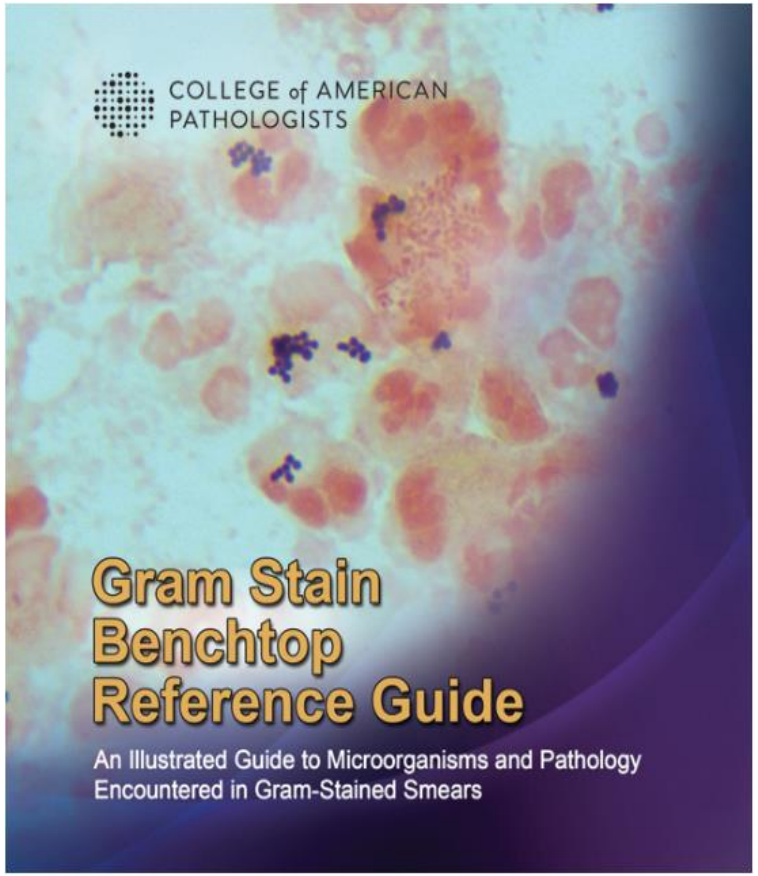


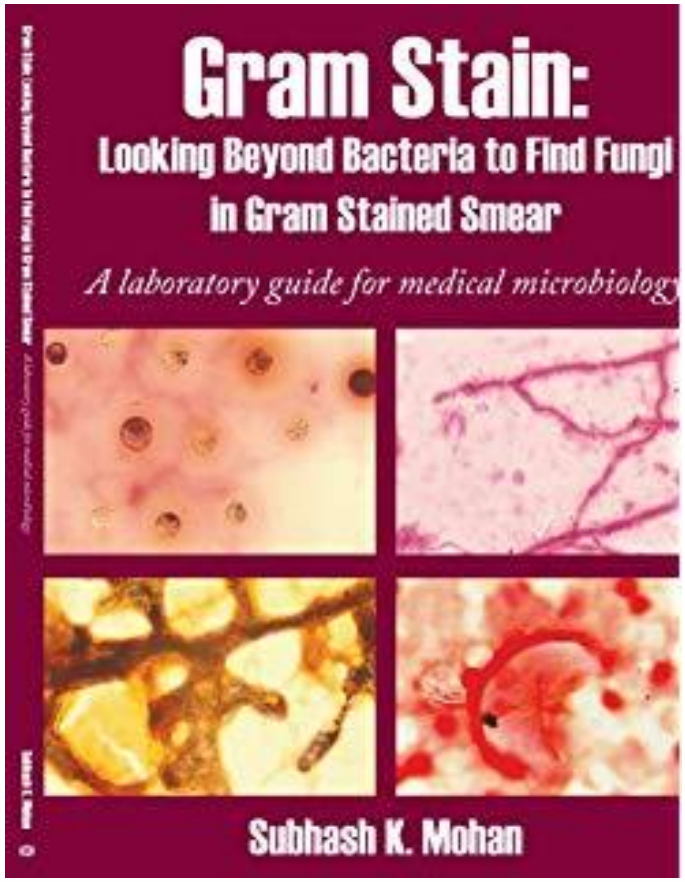
Gram negativo

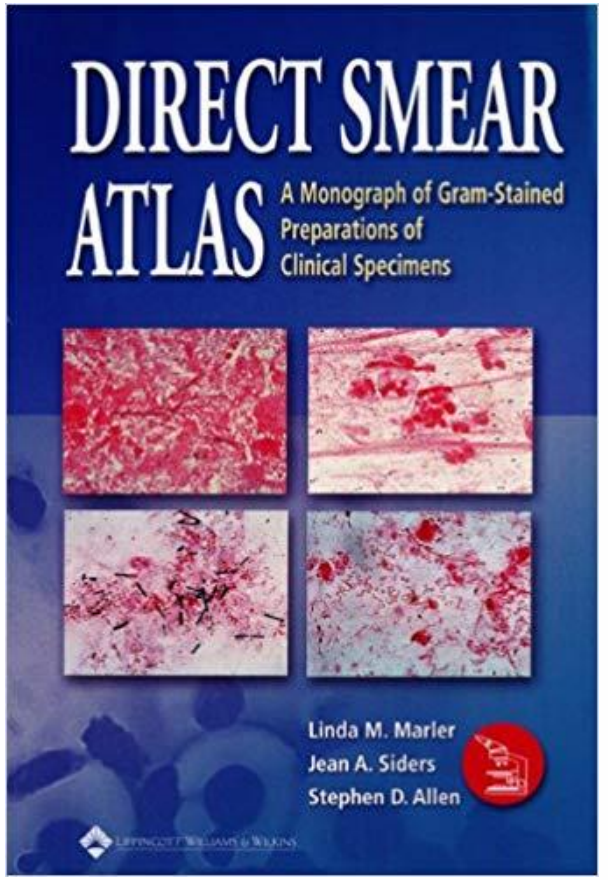


Gram positivo

REFERÊNCIAS

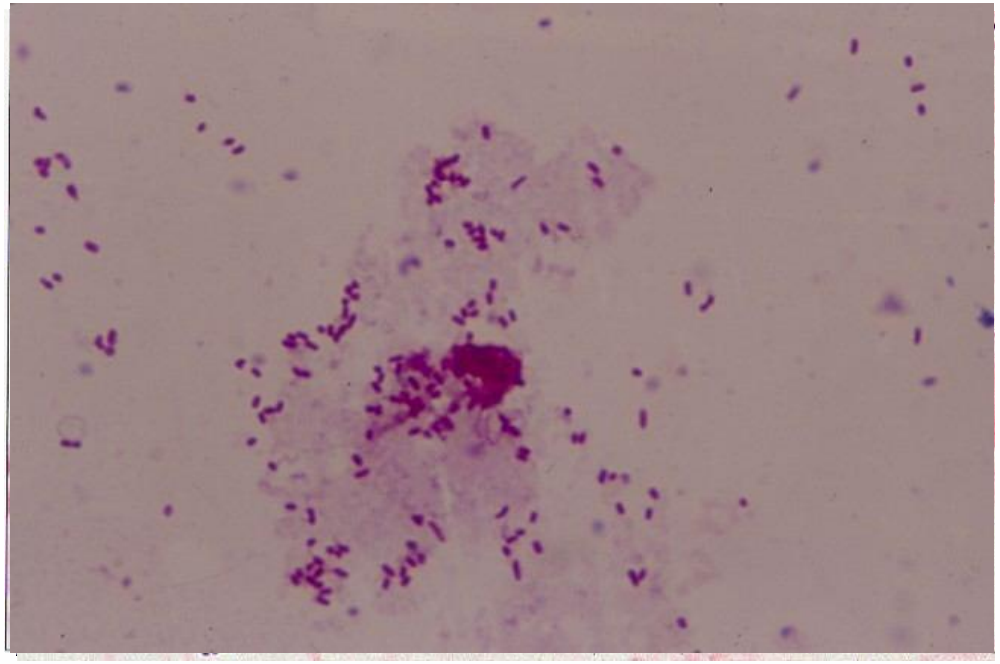






INDICAÇÕES

- ✓ Avaliação da qualidade da amostra
- ✓ Exclusão da hipótese infecciosa bacteriana da doença
- ✓ Auxílio na identificação bacteriana
- ✓ Investigação de antibioticoterapia prévia
- ✓ Predição do prognóstico
- ✓ Emissão de resultado parcial
- ✓ Diagnóstico primário/ precoce
- ✓ Ferramenta para Stewardship
- ✓ Análise criteriosa do crescimento bacteriano
- ✓ Indispensável para análise de resultados discordantes
- ✓ Fundamental para a tomada de decisões



LEITURA

Células - PMNs Média em 10 campos (objetiva10 X)				Bactérias e leveduras Interpretação - Gram (objetiva 100 X)			
0	+	++	+++	+	++	+++	++++
neg	raro	pouco	muito	raras	poucas	muitas	numerosas
0	1-9	10-25	>25	<1	1-5	6-30	>30

Isenberg, 2007

46º Congresso Brasileiro de Análises Clínicas
7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

MICROSCOPISTAS

NÍVEL 1 – Competências mínimas

Morfologia	Microrganismos
Cocos Gram positivos	Estafilococos/ estreptococos
Cocos Gram positivos em cadeias	Estreptococos
Cocos Gram positivos agrupados	Estafilococos
Bacilos Gram positivos	Qualquer bacilo Gram positivo
Cocobacilos Gram negativos	*Haemophilus*
Diplococos Gram negativos	*Neisseria/ Moraxella*
Bacilos Gram negativos	Qualquer bacilo Gram negativo
Leveduras	Candida/ criptococos
Leveduras com pseudo-hifas	Candida (exceto *C. glabrata*)
Cocobacilos Gram variáveis	*Gardnerella vaginalis*

Polimorfonucleares

Células escamosas/ epiteliais

Triagem de escarro

”Clue-cells”

SBAC
Sociedade Brasileira de Análises Clínicas

46º Congresso Brasileiro de Análises Clínicas
7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

MICROSCOPISTAS

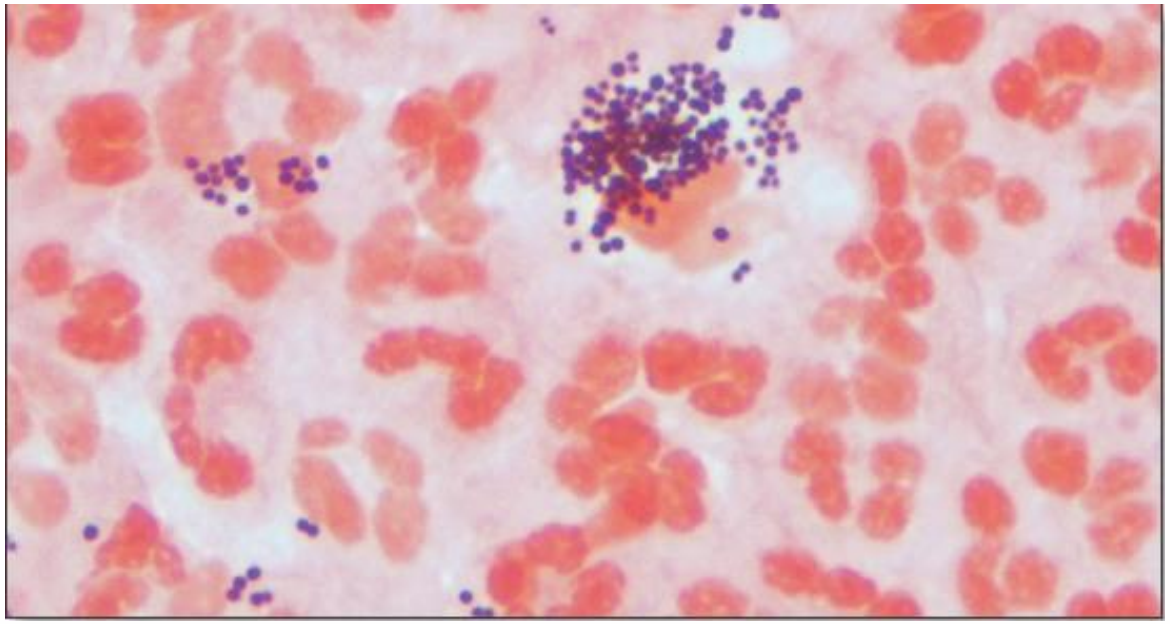
NÍVEL 2 – Competências mínimas

Morfologia	Microrganismos
Diplococos Gram positivos lanceolados	*S. pneumoniae*
Bacilos Gram positivos difteróides	*Corynebacterium*
Bacilos Gram positivos com endosporos	*Bacillus/ Clostridium*
Bacilos Gram positivos filamentosos	*Nocardia/ Actinomyces*
Hifas septadas	*Aspergillus/ Penicillium, ...*
Bacilos Gram negativos grossos	*Enterobactérias*
Bacilos Gram negativos finos	Não-fermentadores
Bacilos Gram negativos pleomórficos	Bacteroides
Diplobacilos Gram negativos	Acinetobacter
Bacilos Gram negativos fusiformes	*Fusobacterium*
Bacilos Gram negativos curvos	*Campylobacter/ Vibrio*
Cocos Gram negativos pequenos	*Brucella*

SBAC
Sociedade Brasileira de Análises Clínicas

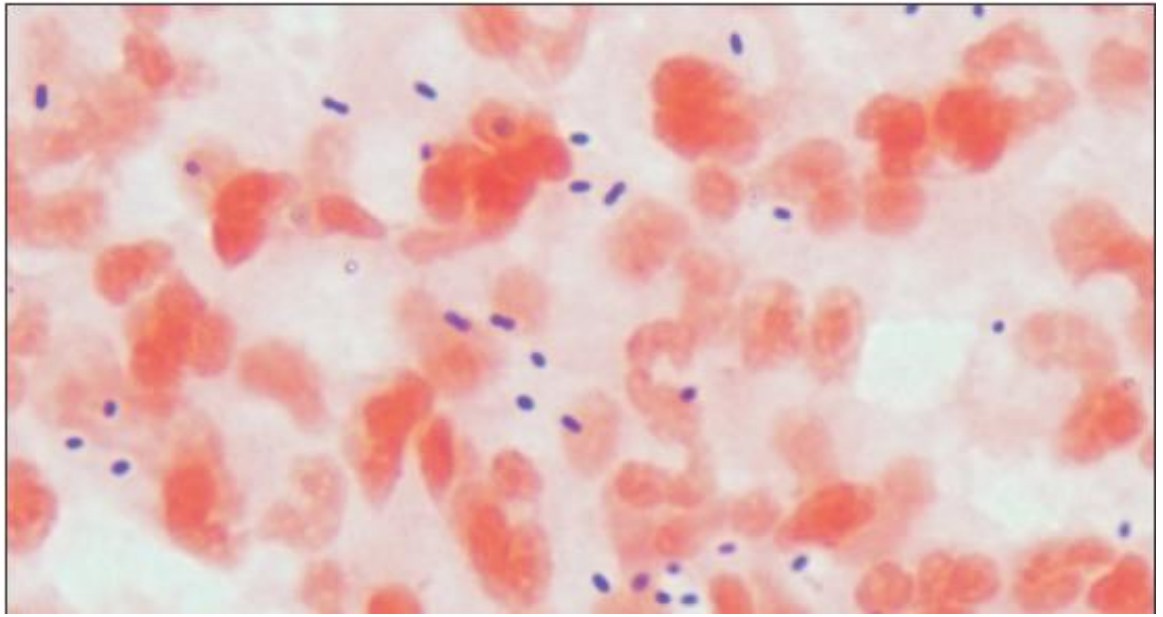
COCOS GRAM +

Líquor



Staphylococcus aureus

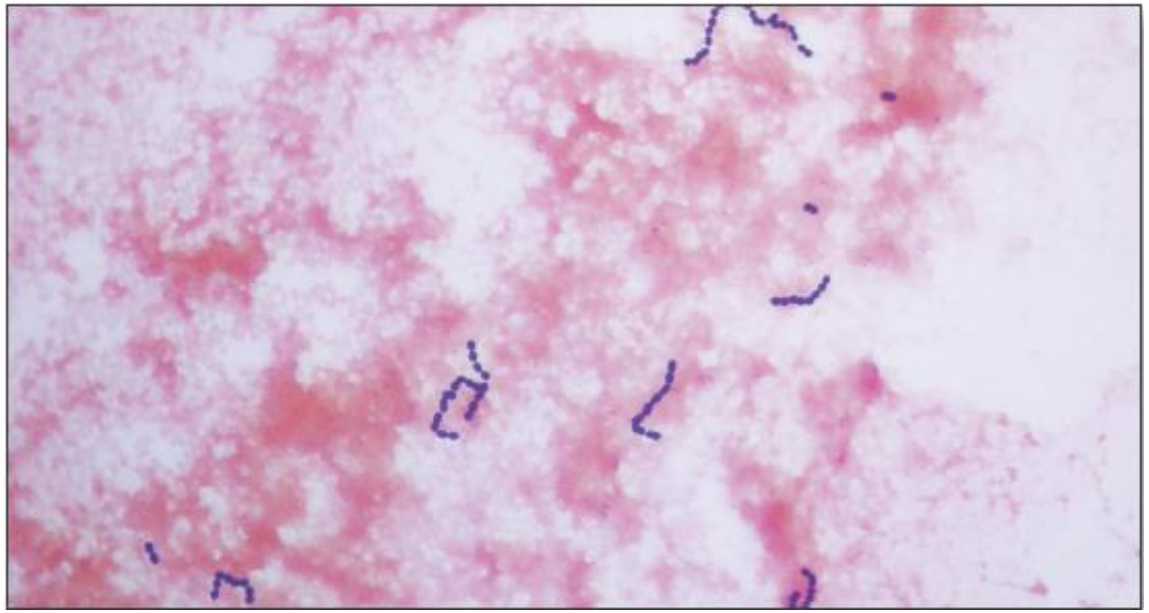
Líquor



Streptococcus pneumoniae

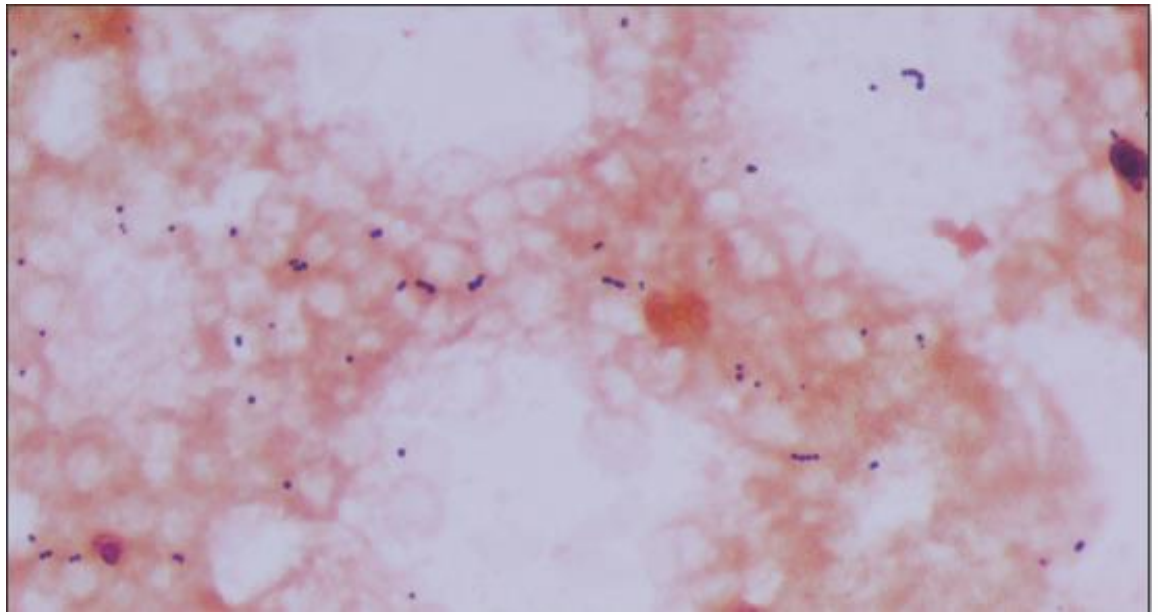
COCOS GRAM +

Hemocultura



Enterococcus faecium

Fluido corporal



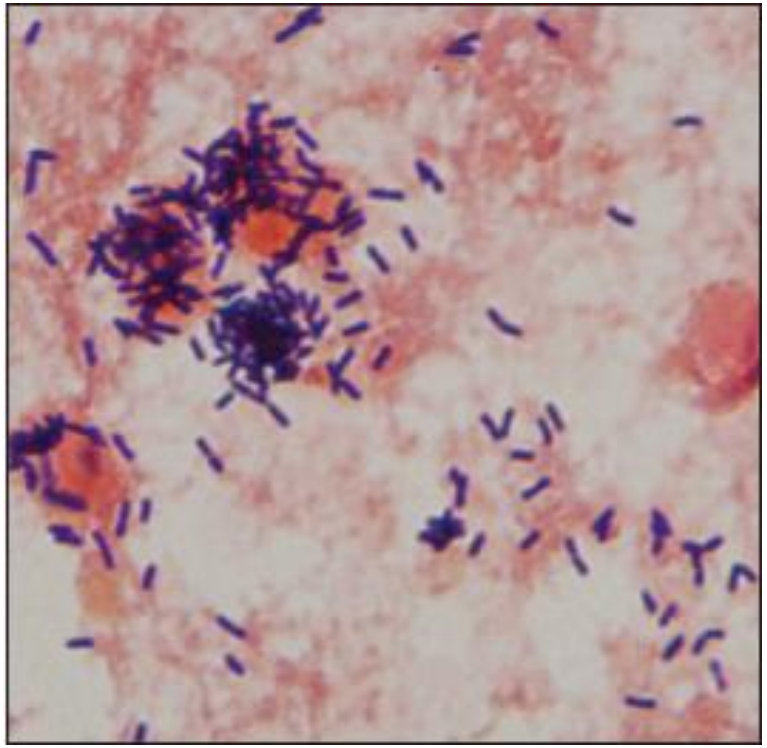
Streptococcus pyogenes

46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

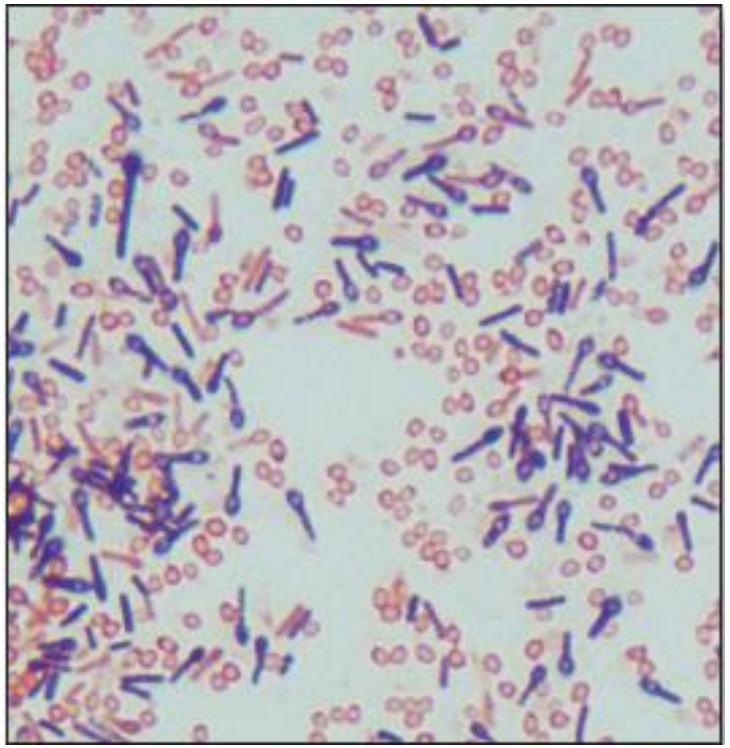
BACILOS GRAM +

Hemocultura



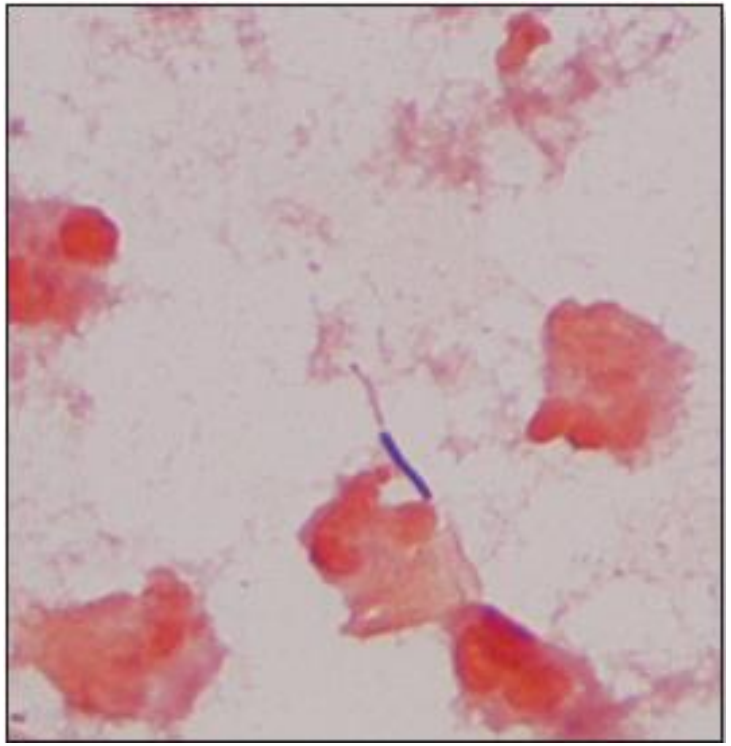
Listeria monocytogenes

Hemocultura



***Bacillus* spp.**

Pé diabético

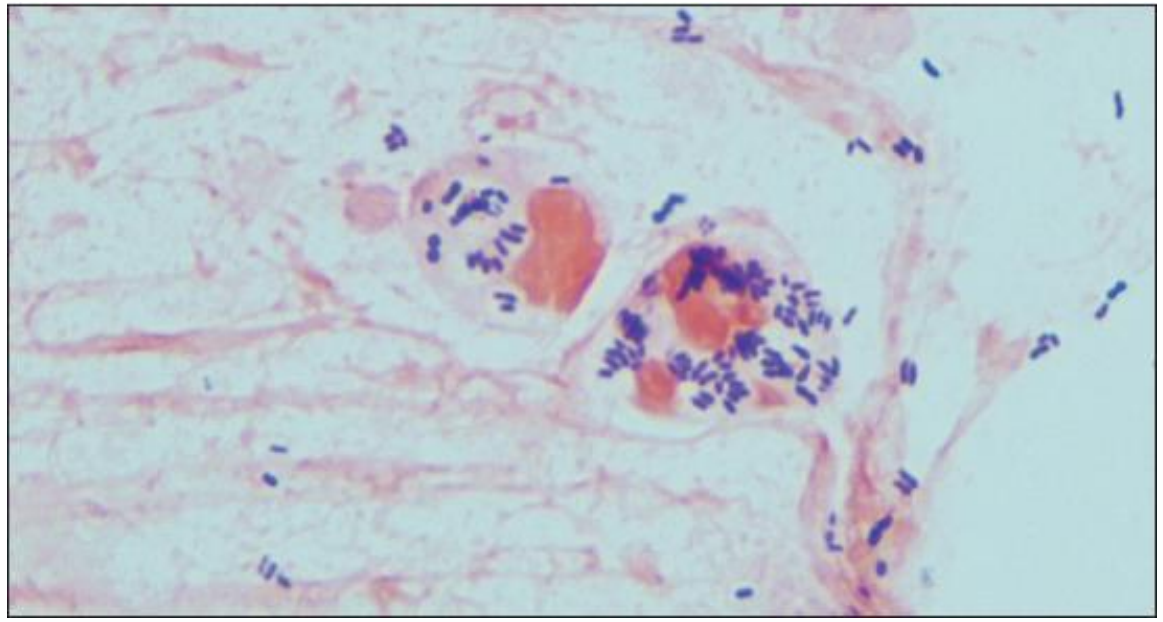


***Clostridium* spp.**

SBAC
Sociedade Brasileira de Análises Clínicas

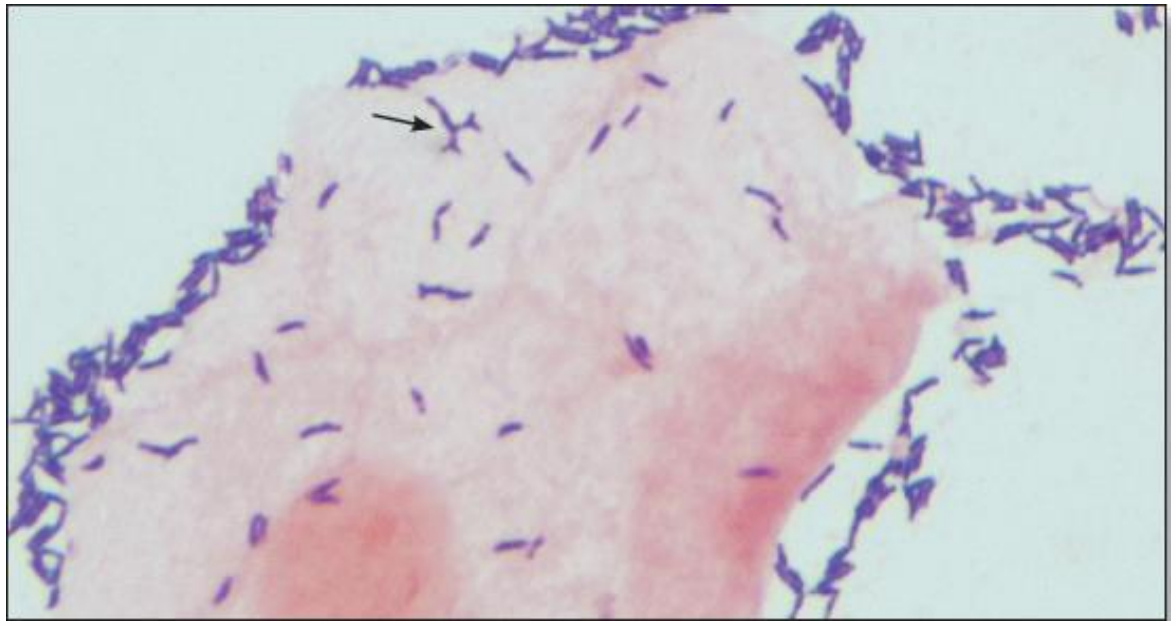
BACILOS GRAM +

Urina



Corynebacterium urealyticum

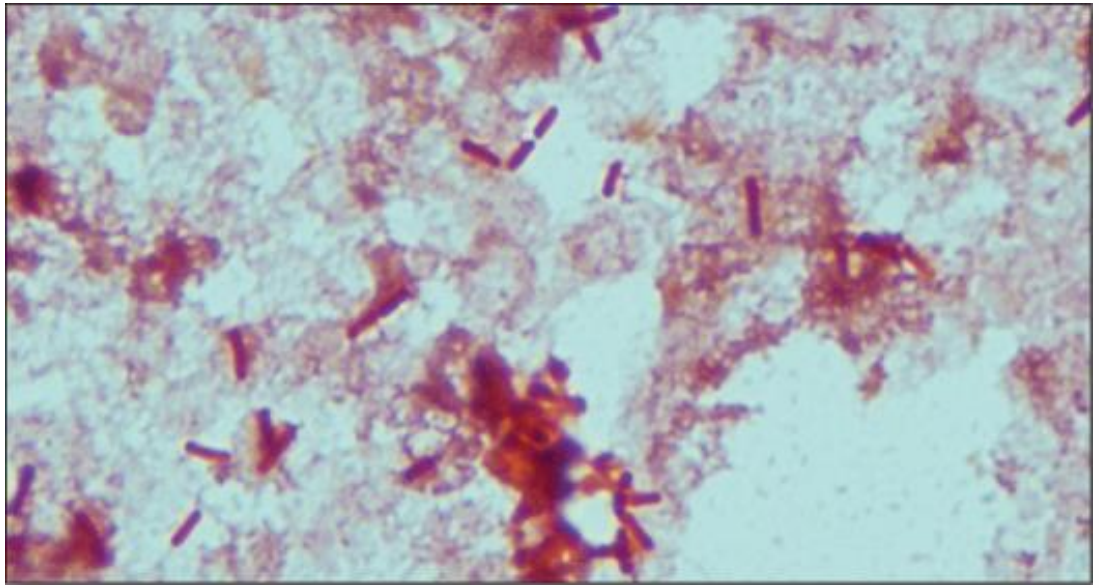
Secreção vaginal



***Bifidobacterium* spp.** ***Lactobacillus* spp.**

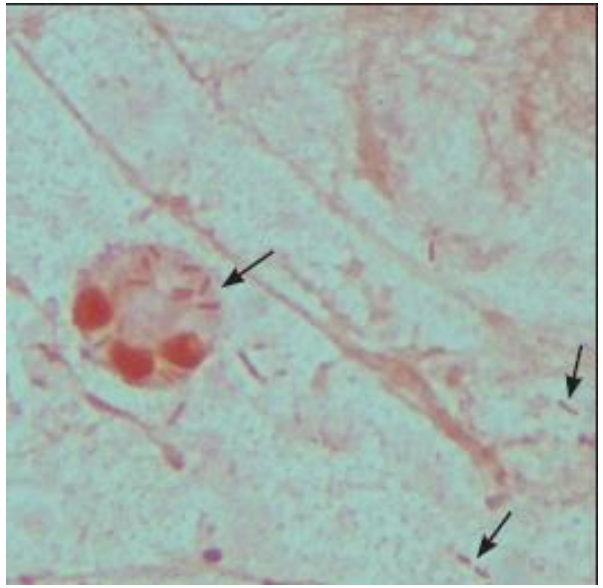
BACILOS GRAM -

Hemocultura



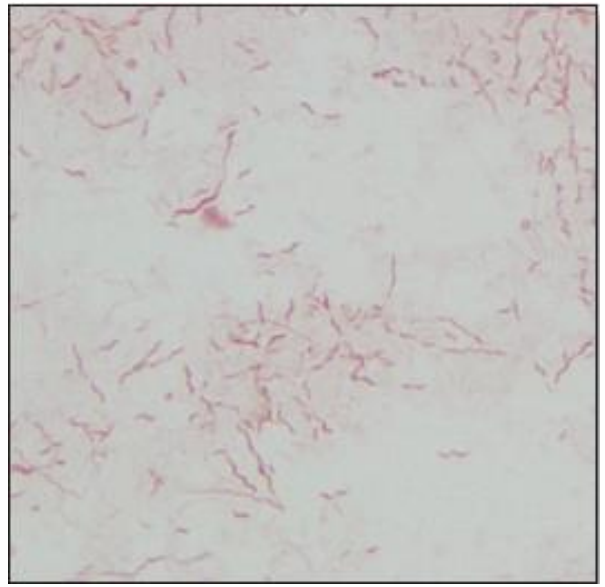
Escherichia coli

Tecido



Pseudomonas aeruginosa

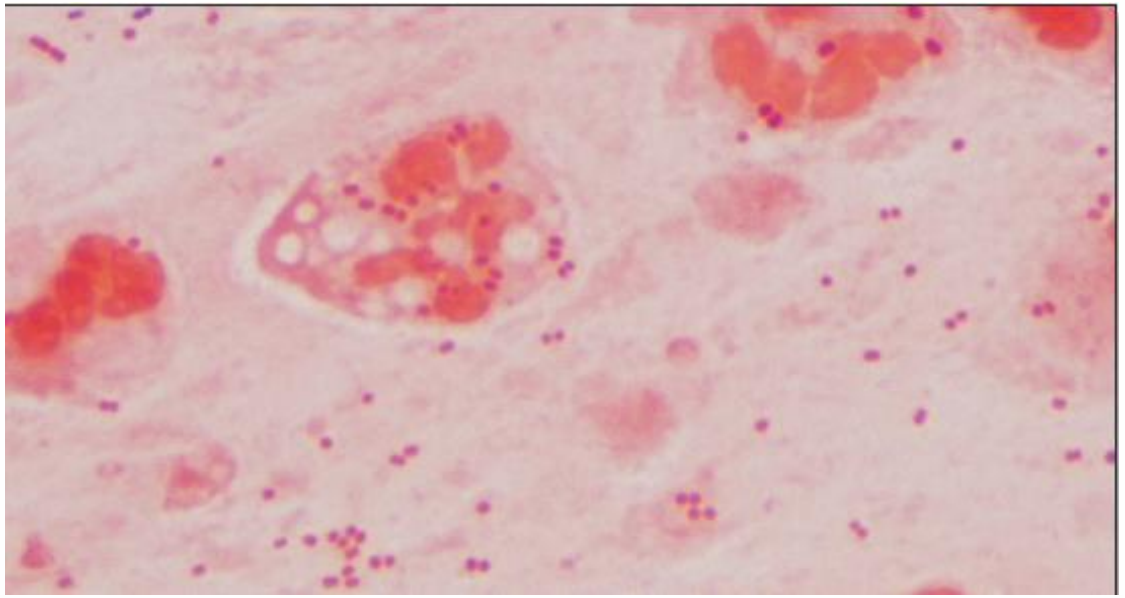
Hemocultura



***Campylobacter* spp.**

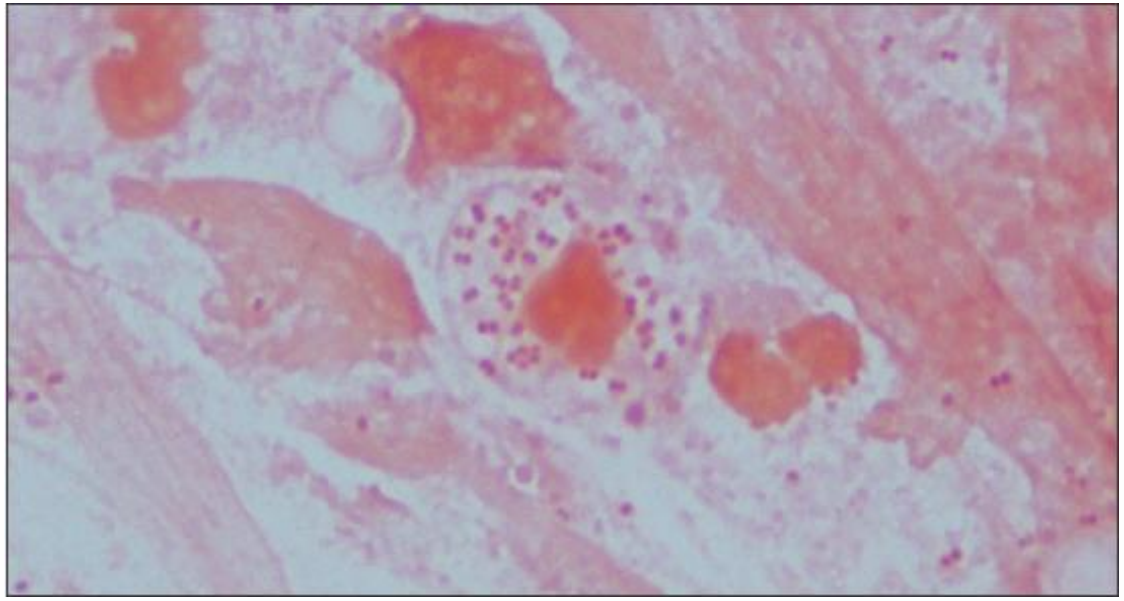
DIPLOCOCOS GRAM -

Escarro



Moraxella catarrhalis

Secreção uretral



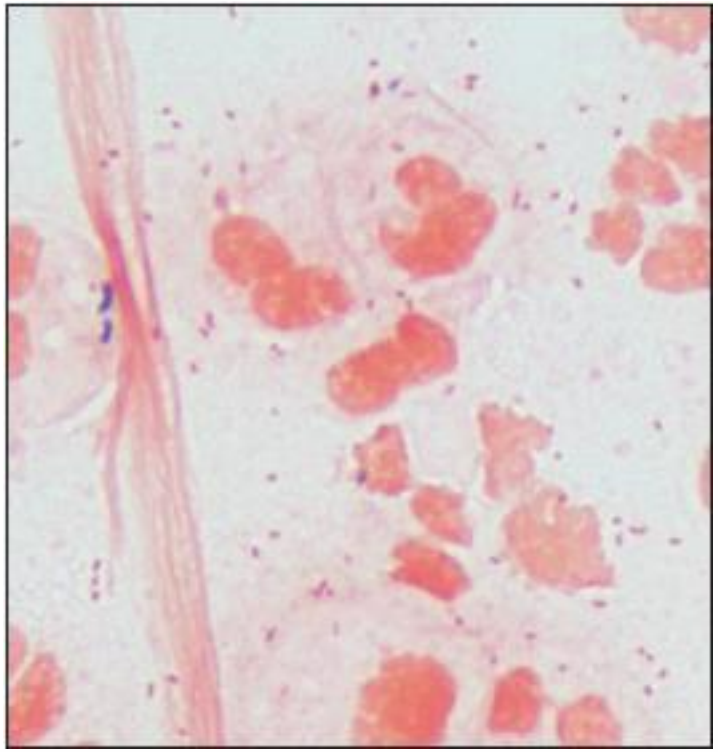
Neisseria gonorrhoeaea

46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

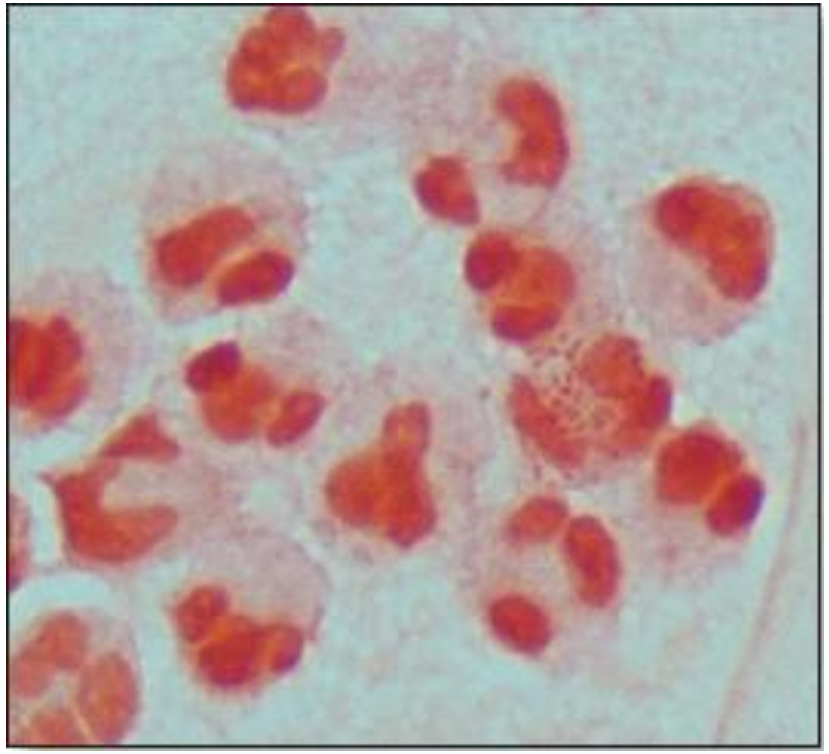
COCOBACILOS GRAM -

Escarro



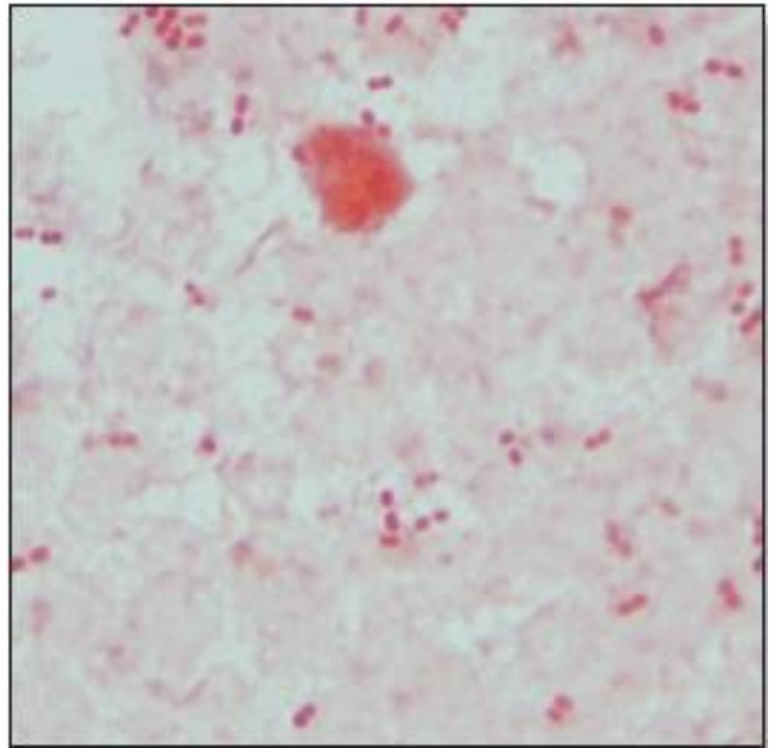
Haemophilus influenzae

Líquido intra-abdominal



***Bacteroides* spp.**

Hemocultura

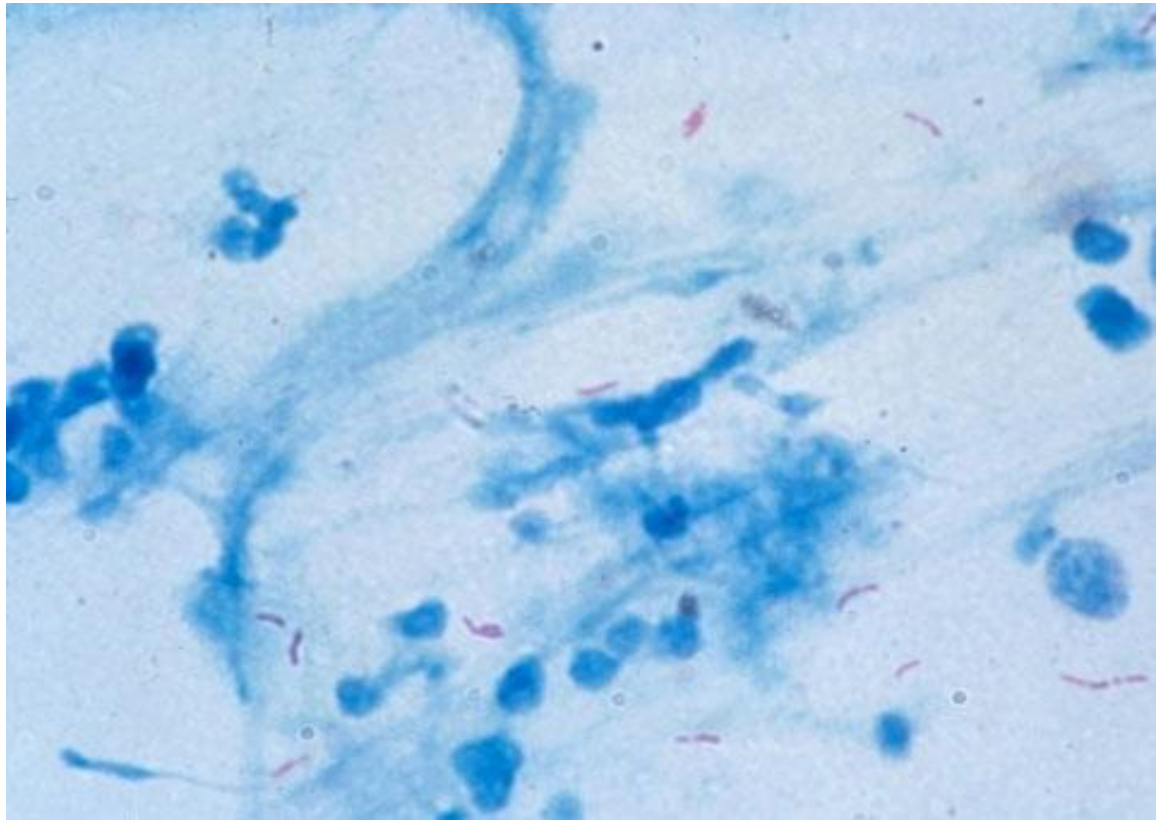


Acinetobacter baumannii

SBAC
Sociedade Brasileira de Análises Clínicas

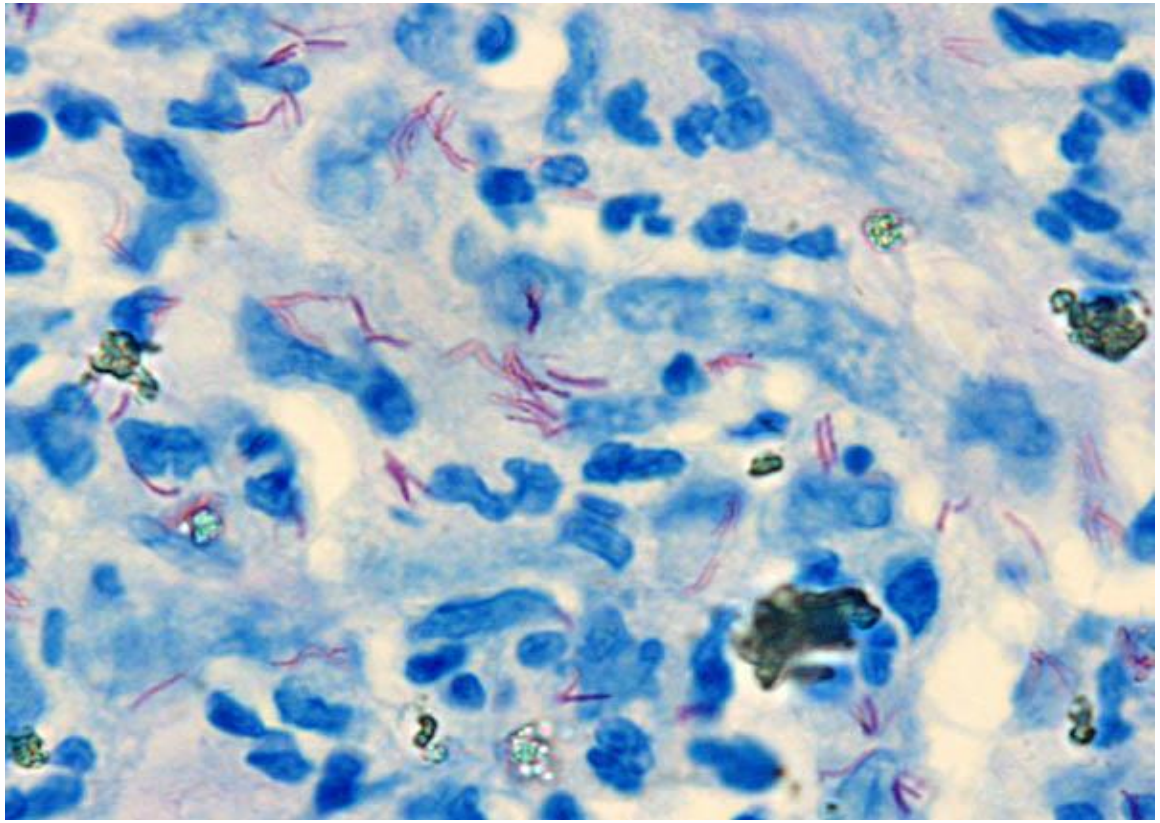
ZIEHL-NEELSEN

Escarro



Mycobacterium tuberculosis

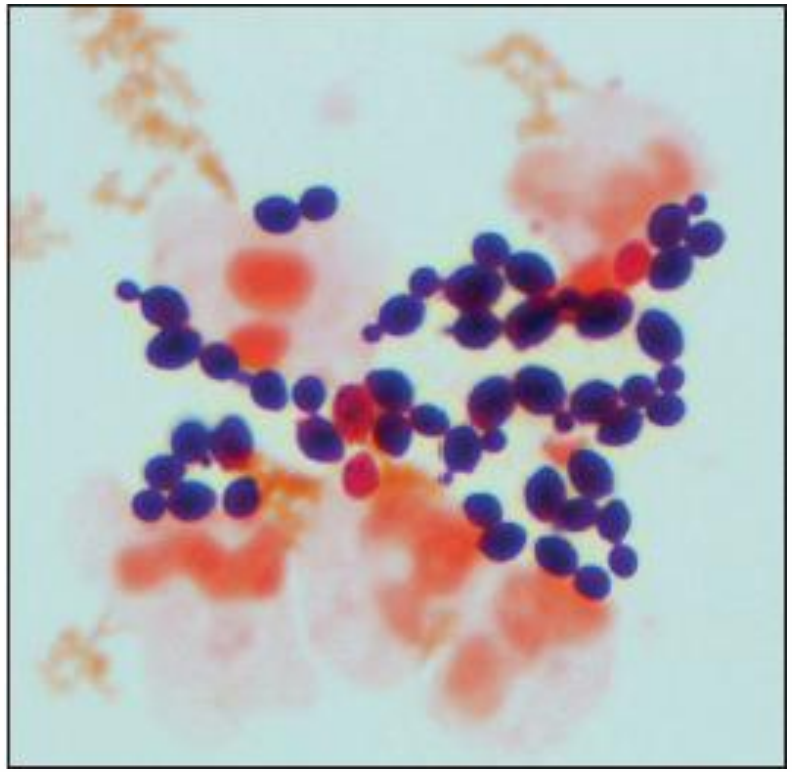
Biópsia de pele



Mycobacterium leprae

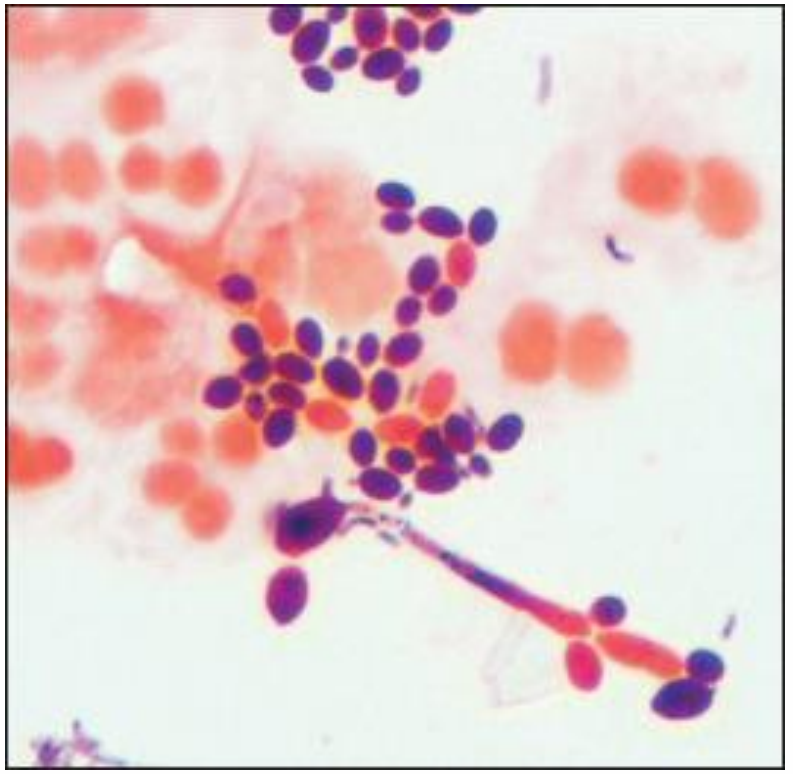
FUNGOS

Líquido peritoneal



Candida glabrata

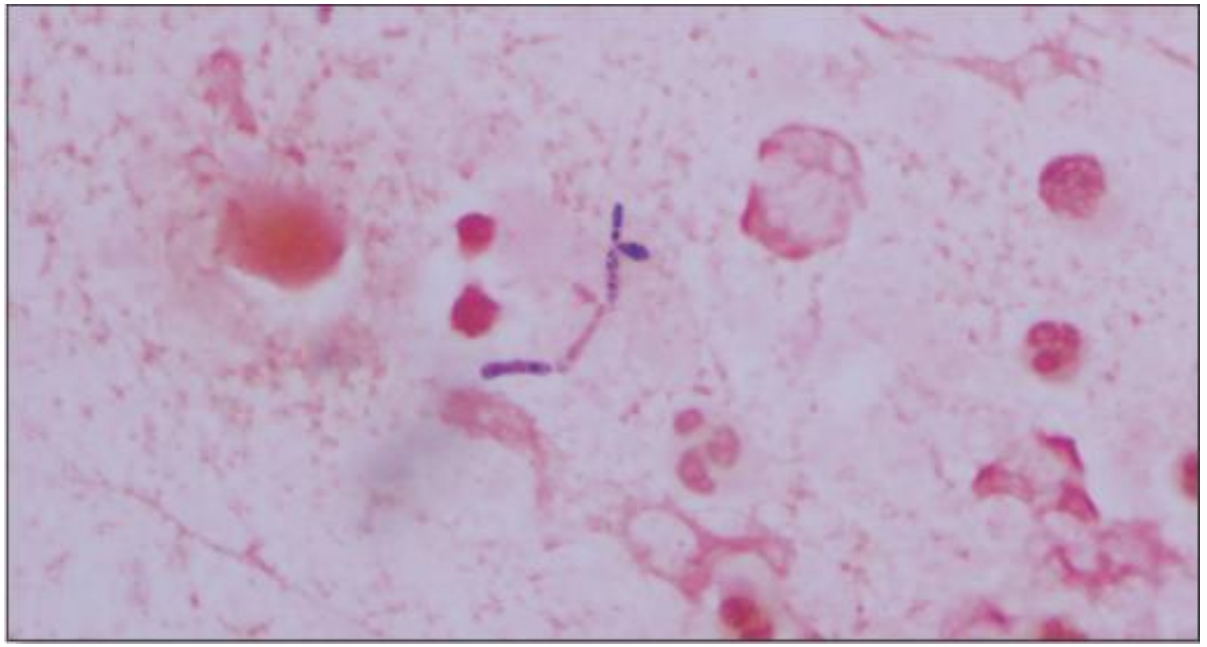
Secreção vaginal



Candida albicans

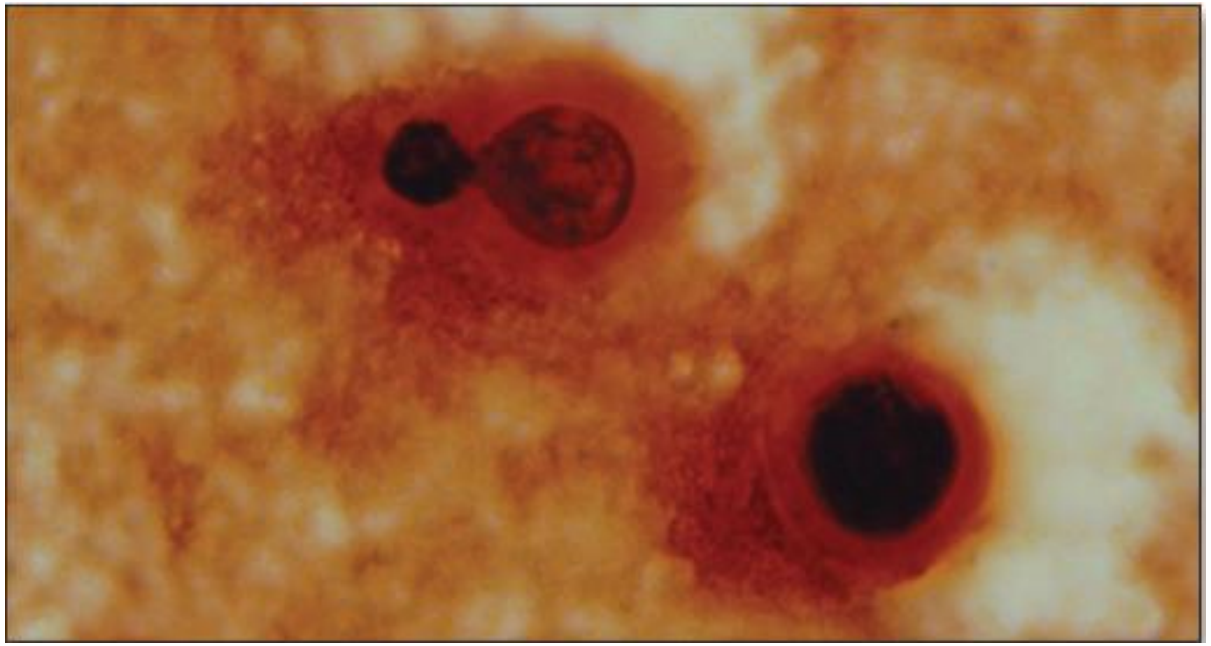
FUNGOS

Líquido biliar



Candida tropicalis

Hemocultura



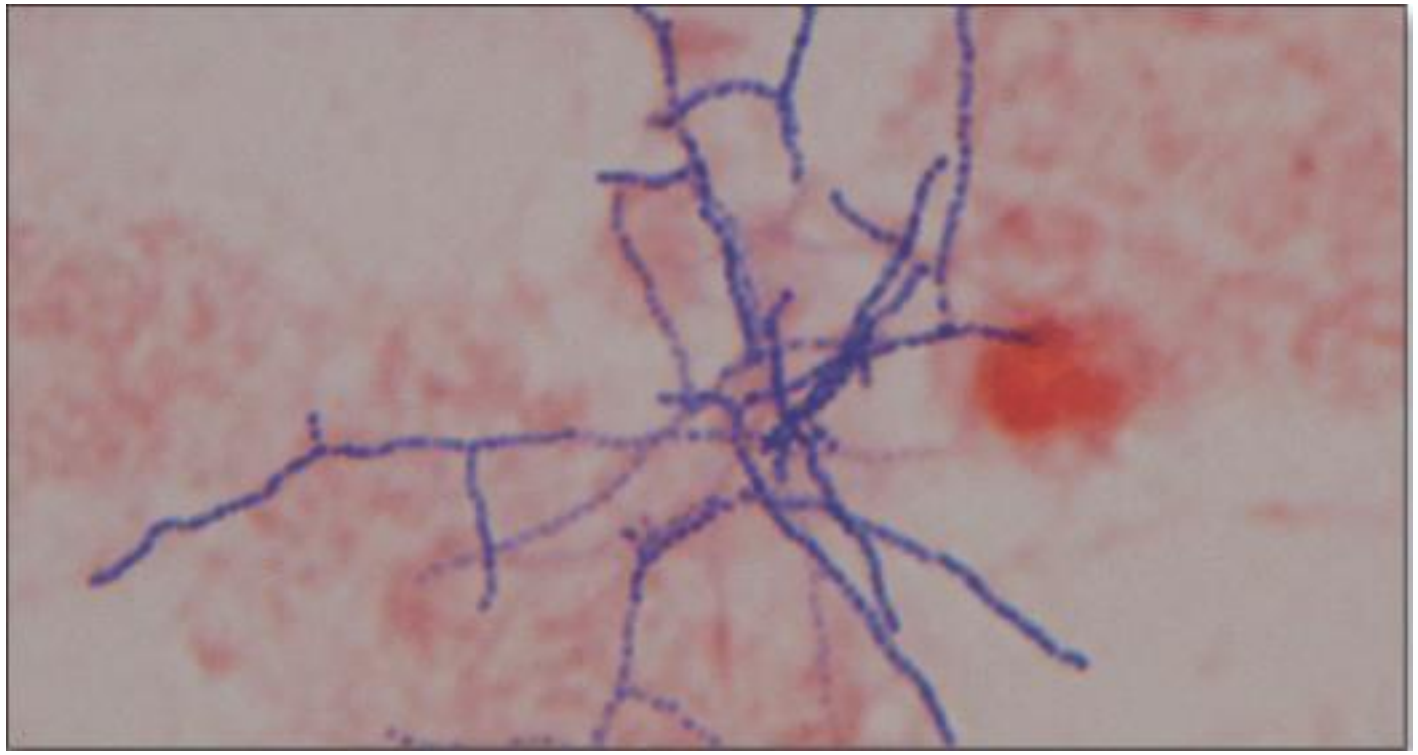
Cryptococcus neoformans

FUNGOS

Escarro

***Aspergillus* spp.**

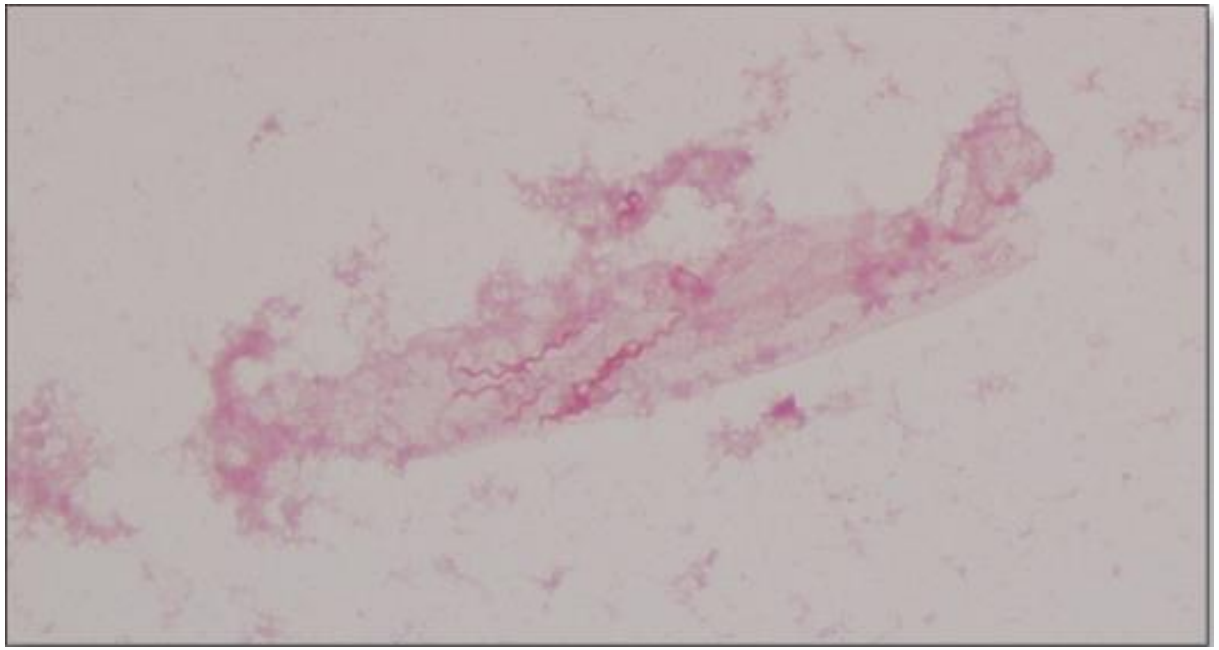
Hemocultura



Nocardia brasiliensis

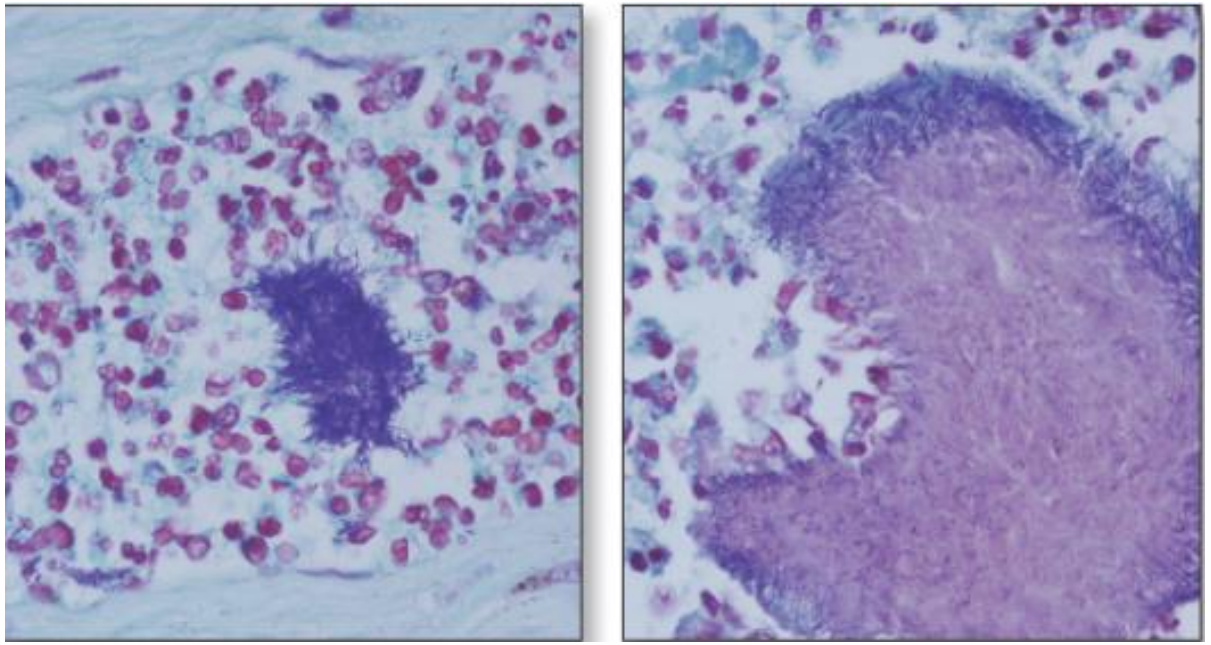
OUTROS

Hemocultura



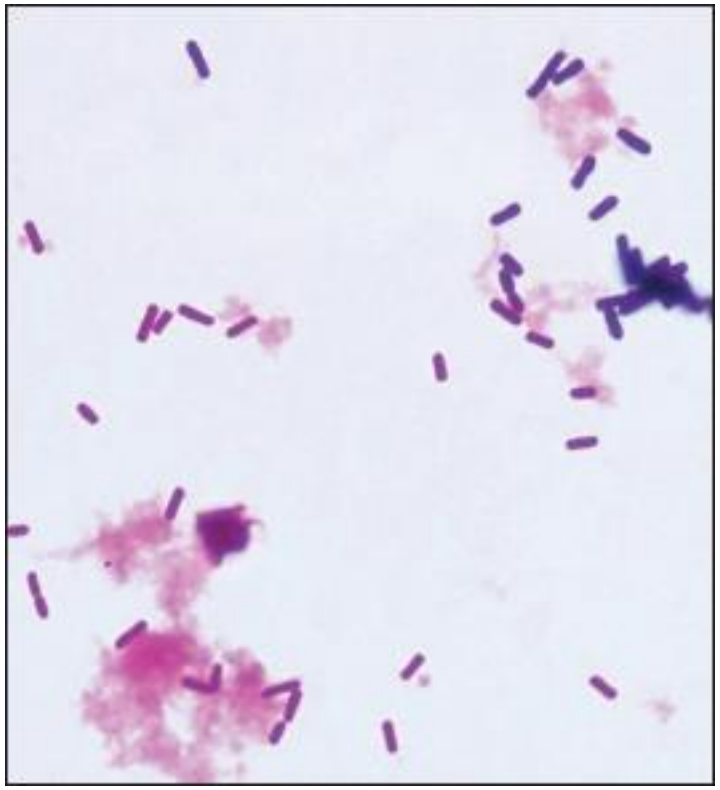
***Leptospira* spp.**

Tecido

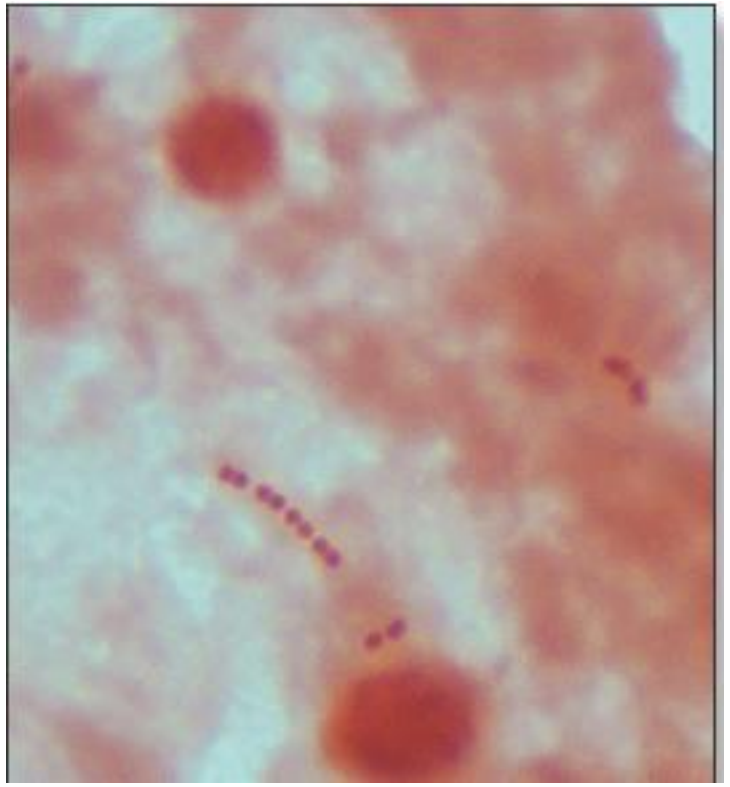


Mycobacterium abscessus

ERROS DE COLORAÇÃO

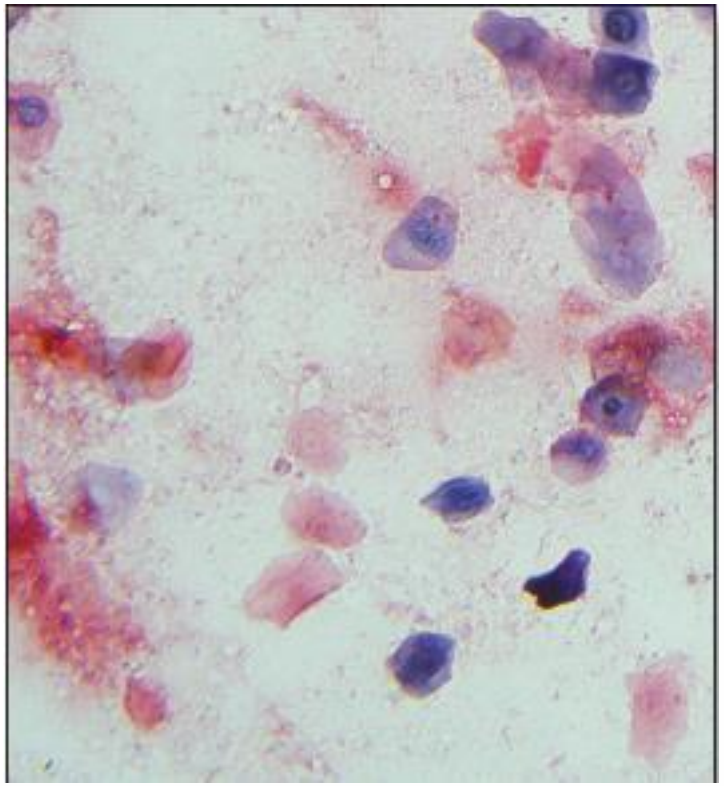


Pouco descoradas

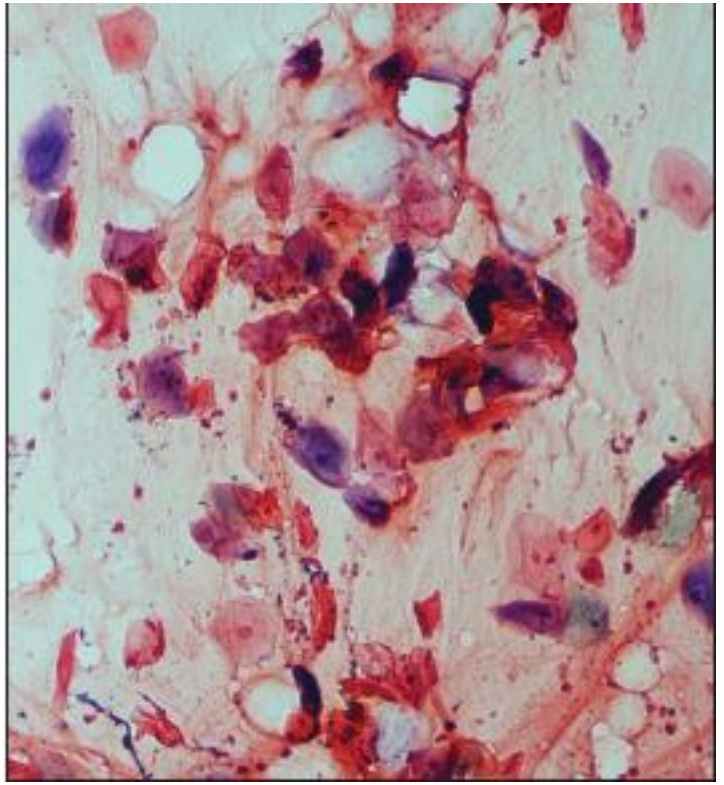


Muito descoradas

AMOSTRAS INADEQUADAS



Amostra de pele contaminada



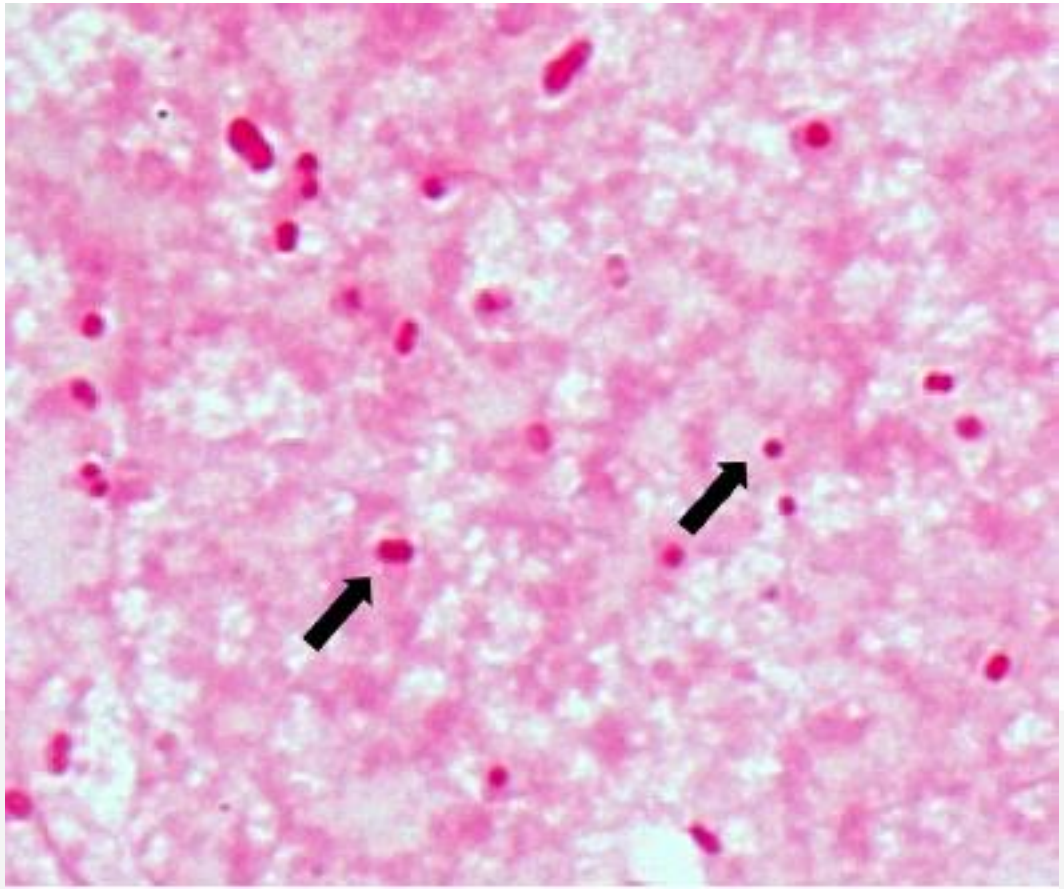
Amostra de escarro contaminada

Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.0787-18
Intern Med 58: 301-305, 2019
http://internmed.jp

[CASE REPORT]

Severe Community-acquired Pneumonia Caused by *Acinetobacter baumannii* Successfully Treated with the Initial Administration of Meropenem Based on the Sputum Gram Staining Findings

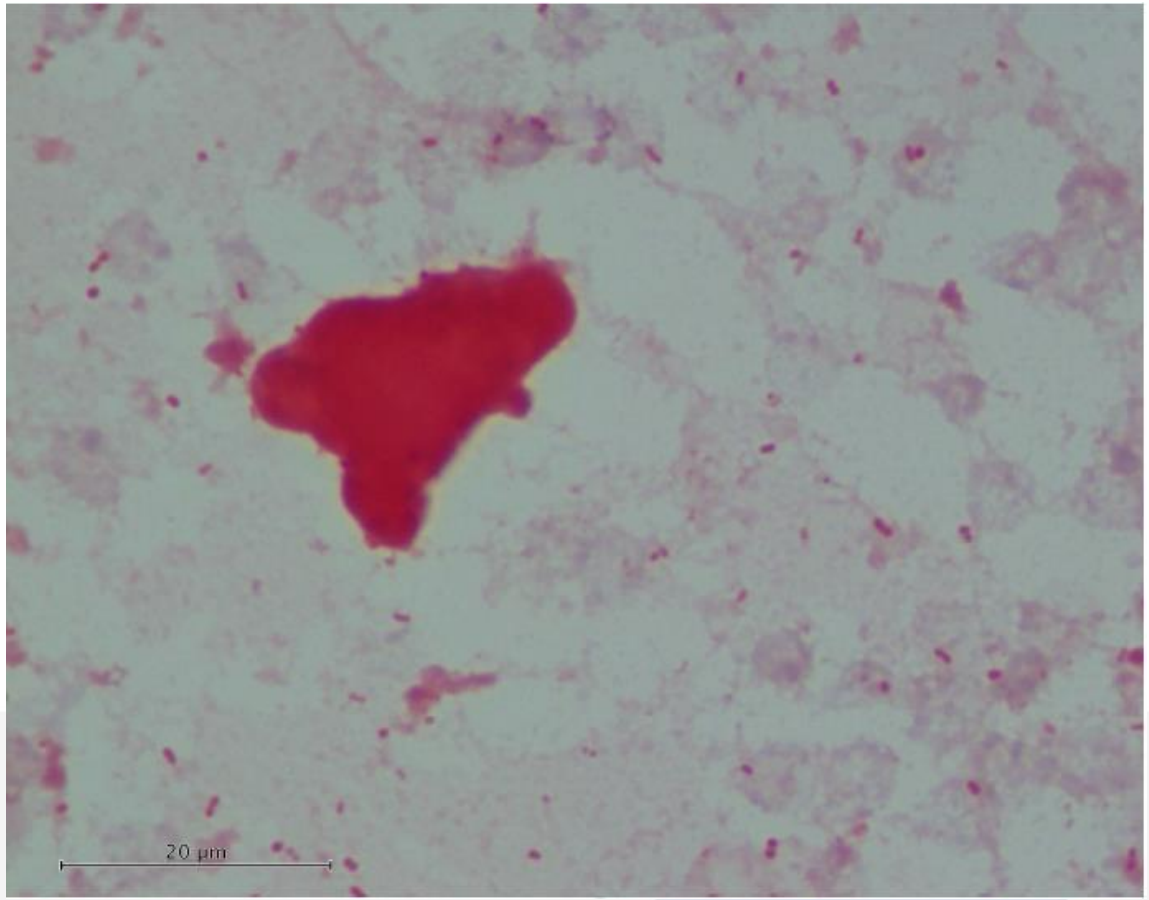
Yurika Iwasawa[1], Naoto Hosokawa[2], Mariko Harada[1], Satoshi Hayano[2], Akihiko Shimizu[2], Daisuke Suzuki[2], Kei Nakashima[3] and Makito Yaegashi[1]



CMI CLINICAL MICROBIOLOGY AND INFECTION
ESCMID OFFICIAL PUBLICATION OF EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Acinetobacter ursingii masquerading as Gram-positive cocci

August 2018 Volume 24, Issue 8, Pages 856–857



Received: 30 May 2018 | Revised: 5 August 2018 | Accepted: 14 September 2018
DOI: 10.1002/jgf2.212

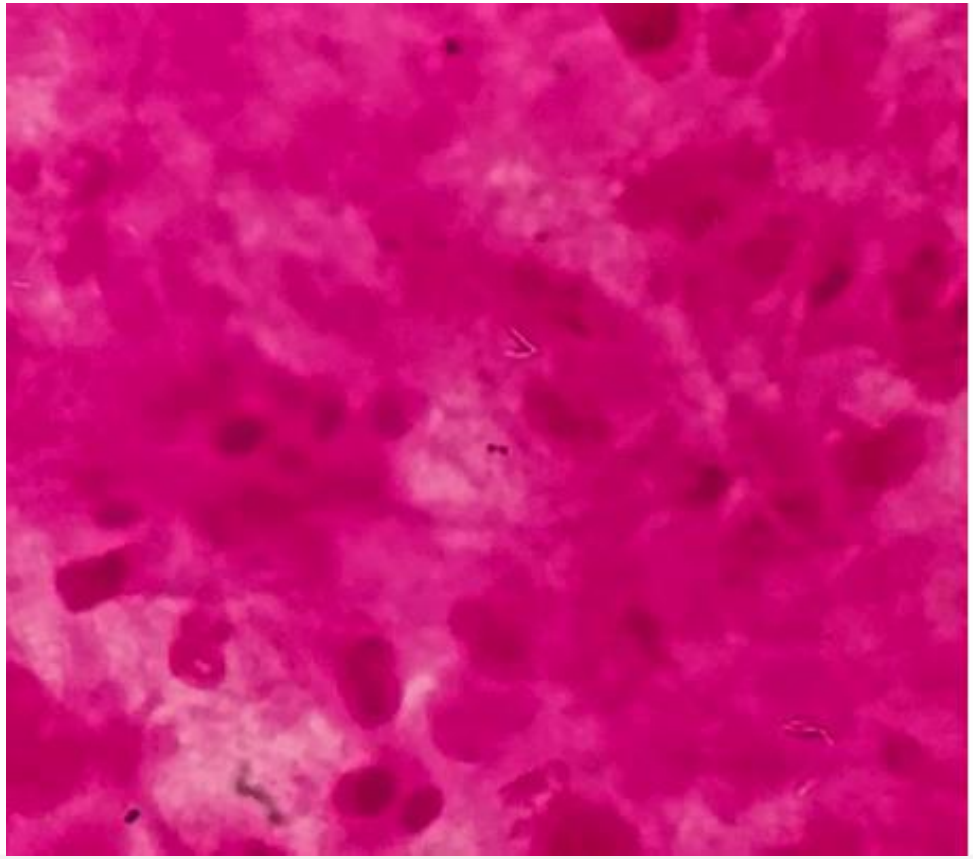
Journal of General and Family Medicine Open Access WILEY

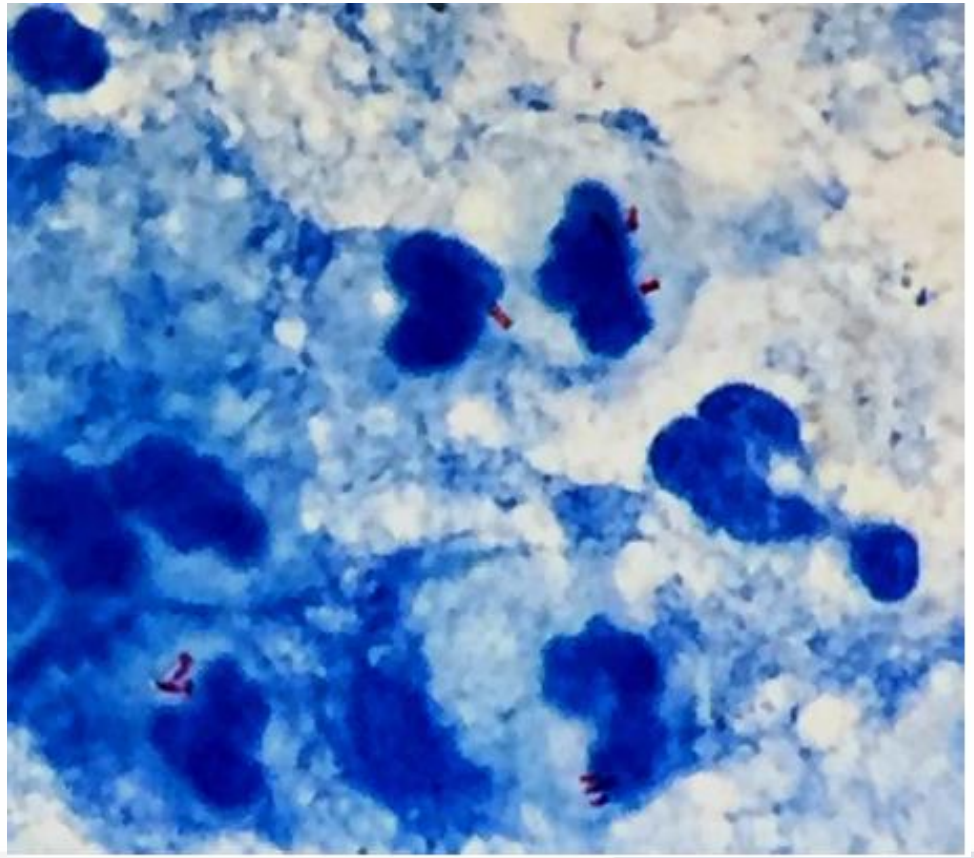
IMAGES IN CLINICAL MEDICINE

Gram-ghost bacilli

Hirokazu Kuroda MD | Naoto Hosokawa MD, PhD

Department of Infectious Diseases, Kameda Medical Center, Kamogawa, Japan





46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

IDCases 7 (2017) 47

Contents lists available at ScienceDirect

IDCases

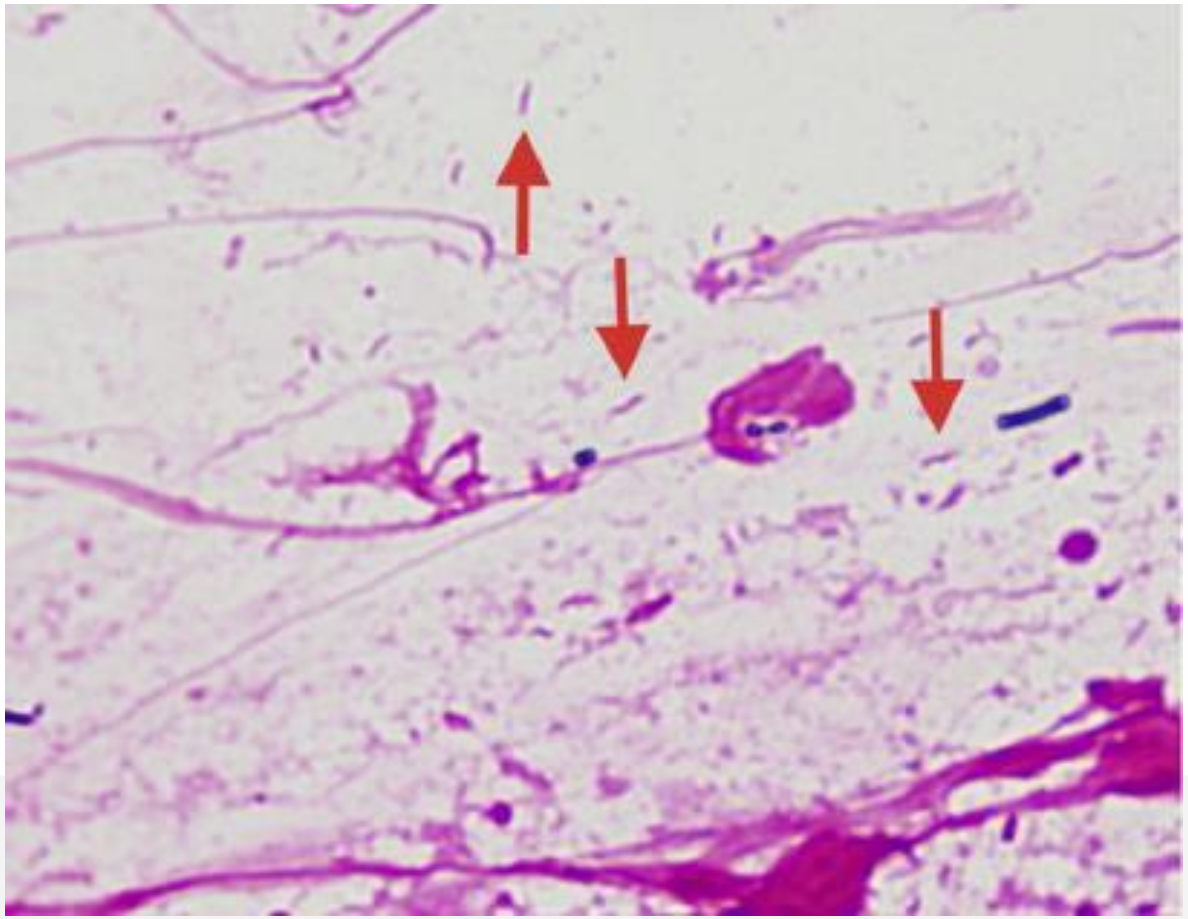
journal homepage: www.elsevier.com/locate/idcr

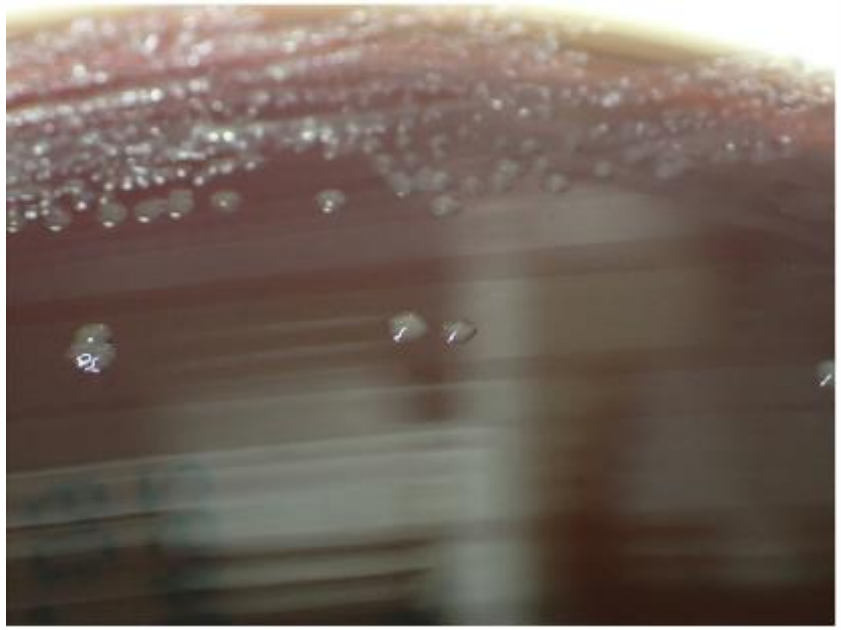
Case report

Campylobacter enterocolitis diagnosed by stool Gram stain

Hidenori Nakagawa*, Masato Nishihara

Department of Pediatrics, Yodogawa Christian Hospital, Japan





SBAC Sociedade Brasileira de Análises Clínicas

Clinical Microbiology and Infection xxx (2018) 1–2

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Picture of a Microorganism

Puzzling mosaics in cerebrospinal fluid

M. Puges [1,*], F. Gabriel [2], M. Carrer [1], S. Perez [1], H. Boijout [3], H. Dutronc [1], C. Cazanave [1]

[1] CHU Bordeaux, Service des maladies infectieuses et tropicales, Bordeaux, France
[2] CHU Bordeaux, Service de parasitologie-mycologie, Bordeaux, France
[3] CHU Bordeaux, Service de bactériologie, Bordeaux, France

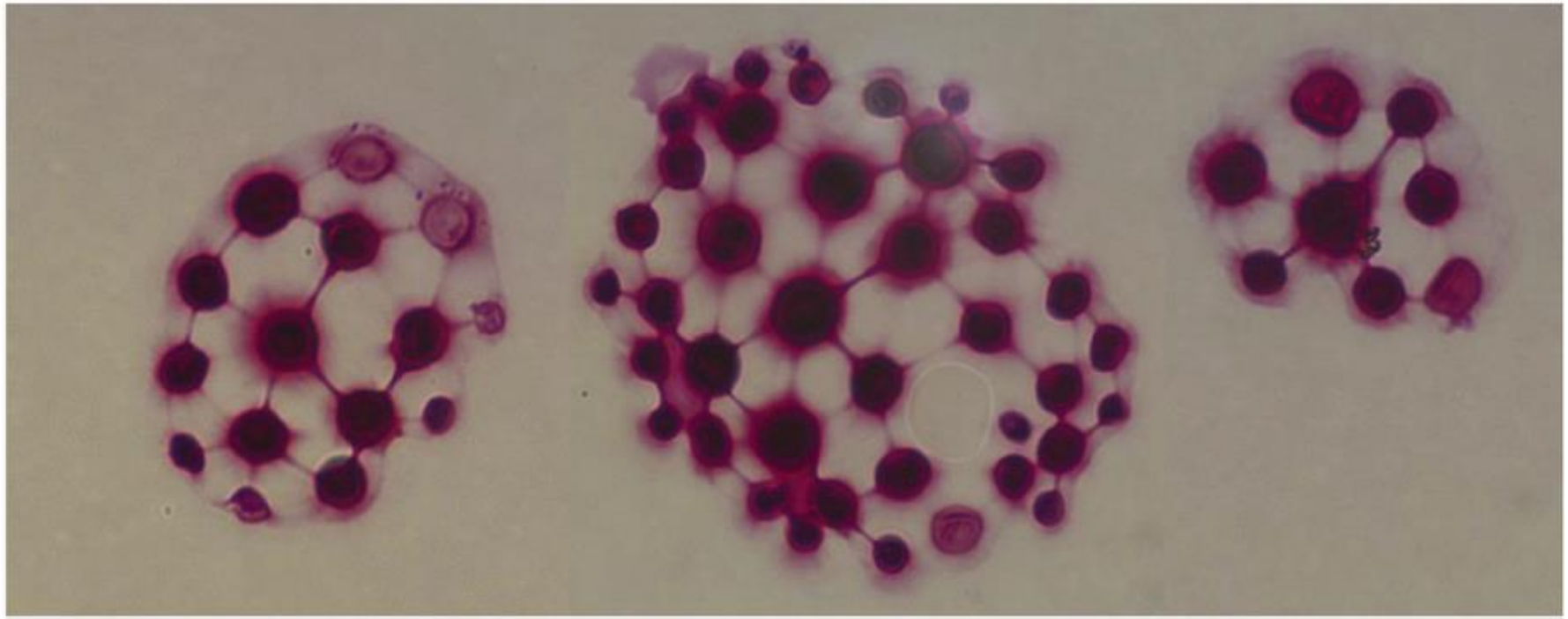


Fig. 1. Confusing Gram staining of cerebrospinal fluid demonstrating 'Crystalloid' structures observed in a *Cryptococcus neoformans* meningitis.

Taniguchi et al. BMC Infectious Diseases (2015) 15:458
DOI 10.1186/s12879-015-1203-6

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

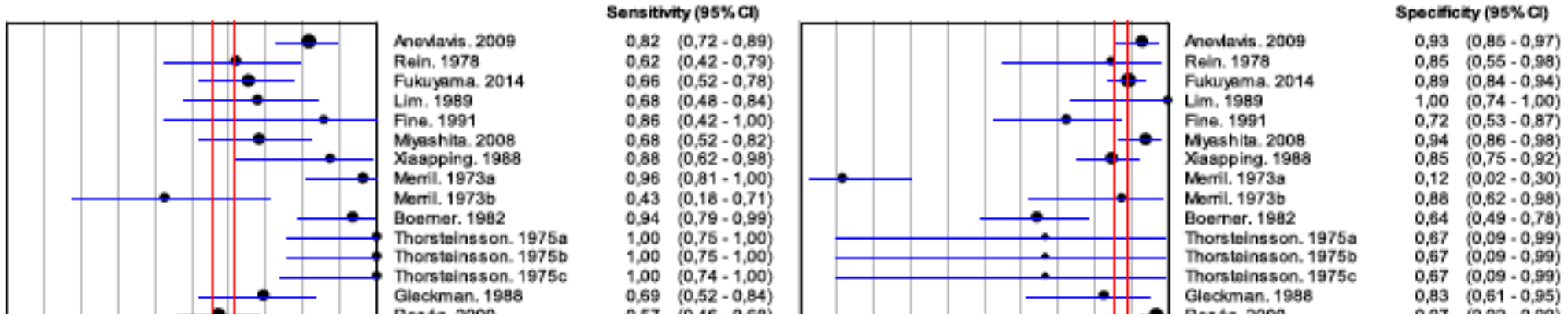
Gram-stain-based antimicrobial selection reduces cost and overuse compared with Japanese guidelines

Tomohiro Taniguchi*, Sanefumi Tsuha, Soichi Shiiki and Masashi Narita

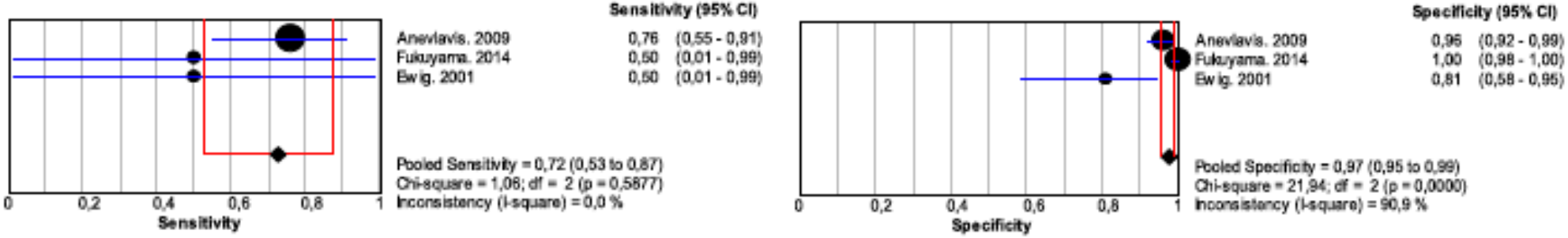
Table 5 Antimicrobial costs in each group

		Antibiotics total cost (yen)		
	N	Gram stain	Guidelines	Cost ratio
Pulmonary system	Subtotal = 45			
Community-acquired pneumonia	7	94,843	115,528	0.82
Aspiration pneumonia	38	635,846	1,940,352	0.33
Urinary tract	Subtotal = 105			
Pyelonephritis	20	447,282	764,816	0.58
Complicated pyelonephritis	67	2,021,718	4,559,744	0.44
Urosepsis	8	484,687	591,654	0.82
Prostatitis	5	100,652	142,056	0.71
Catheter related pyelonephritis	5	202,330	430,245	0.47
Skin & soft tissue	Subtotal = 58			
Cellulitis	34	555,997	1,279,216	0.43
Severe cellulitis	18	540,940	1,505,790	0.36
MRSA suspected cellulitis	6	324,756	1,565,758	0.21
Total	208	5,409,051	12,894,159	0.42

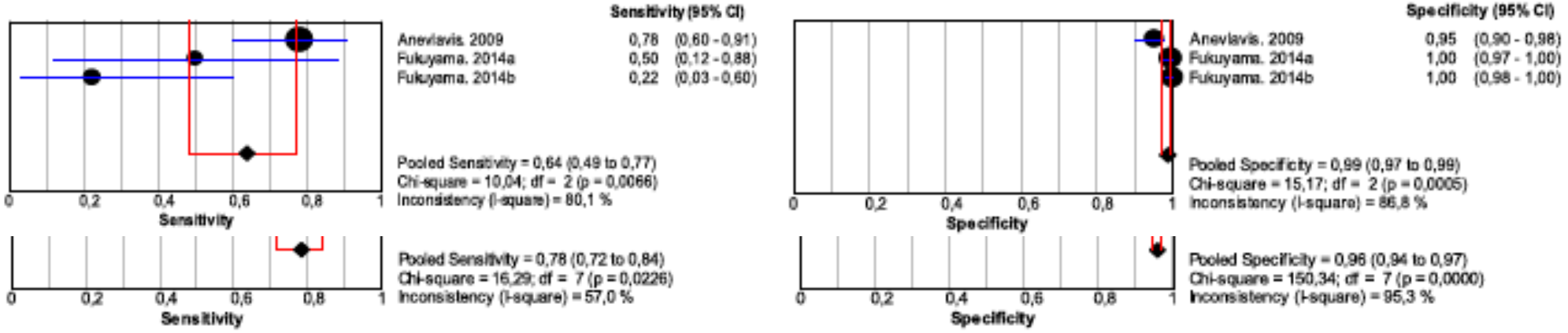
A *Streptococcus pneumoniae*



C *Staphylococcus aureus*



D Gram-negative bacilli



SBAC
Sociedade Brasileira de Análises Clínicas

46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

MAYO CLINIC

ORIGINAL ARTICLE

Meningitis With a Negative Cerebrospinal Fluid Gram Stain in Adults: Risk Classification for an Adverse Clinical Outcome

Nabil T. Khoury, MD; Md Monir Hossain, PhD; Susan H. Wootton, MD; Lucrecia Salazar, MD; and Rodrigo Hasbun, MD, MPH

 ■ Mayo Clin Proc. 2012;87(12):1181-1188

TABLE 2. Etiologies and Outcomes in 567 Patients With Meningitis and a Negative Cerebrospinal Fluid Gram Stain[a]

Etiology	No. (%) of patients	No. (%) of adverse clinical outcomes[b]
Unknown	408 (72)	19 (5)
Urgent treatable	74 (13)	27 (36)
Bacterial meningitis[c]	22	
Cryptococcus neoformans	14	
Herpes simplex virus encephalitis	13	
Varicella zoster virus	5	
Mycobacterium tuberculosis	5	
CNS lymphoma/carcinomatosis	3	
CNS vasculitis	3	
Escherichia coli urosepsis	1	
Toxoplasma gondii	1	
Brain abscess	1	
Cytomegalovirus encephalitis	1	
Rubella encephalitis	1	
Histoplasmosis	1	
Subarachnoid bleed	1	
Epidural abscess	1	
Infective endocarditis	1	
Untreatable	44 (8)	14 (32)
West Nile virus	29	
Enterovirus	11	
St Louis encephalitis virus	3	
Epstein-Barr virus	1	
Nonurgent treatable	41 (7)	0 (0)
Mollaret meningitis	34	
Acute HIV	3	
Neurosyphilis	1	
Multiple sclerosis	1	
Influenza virus type A	1	
Infectious mononucleosis due to cytomegalovirus	1	

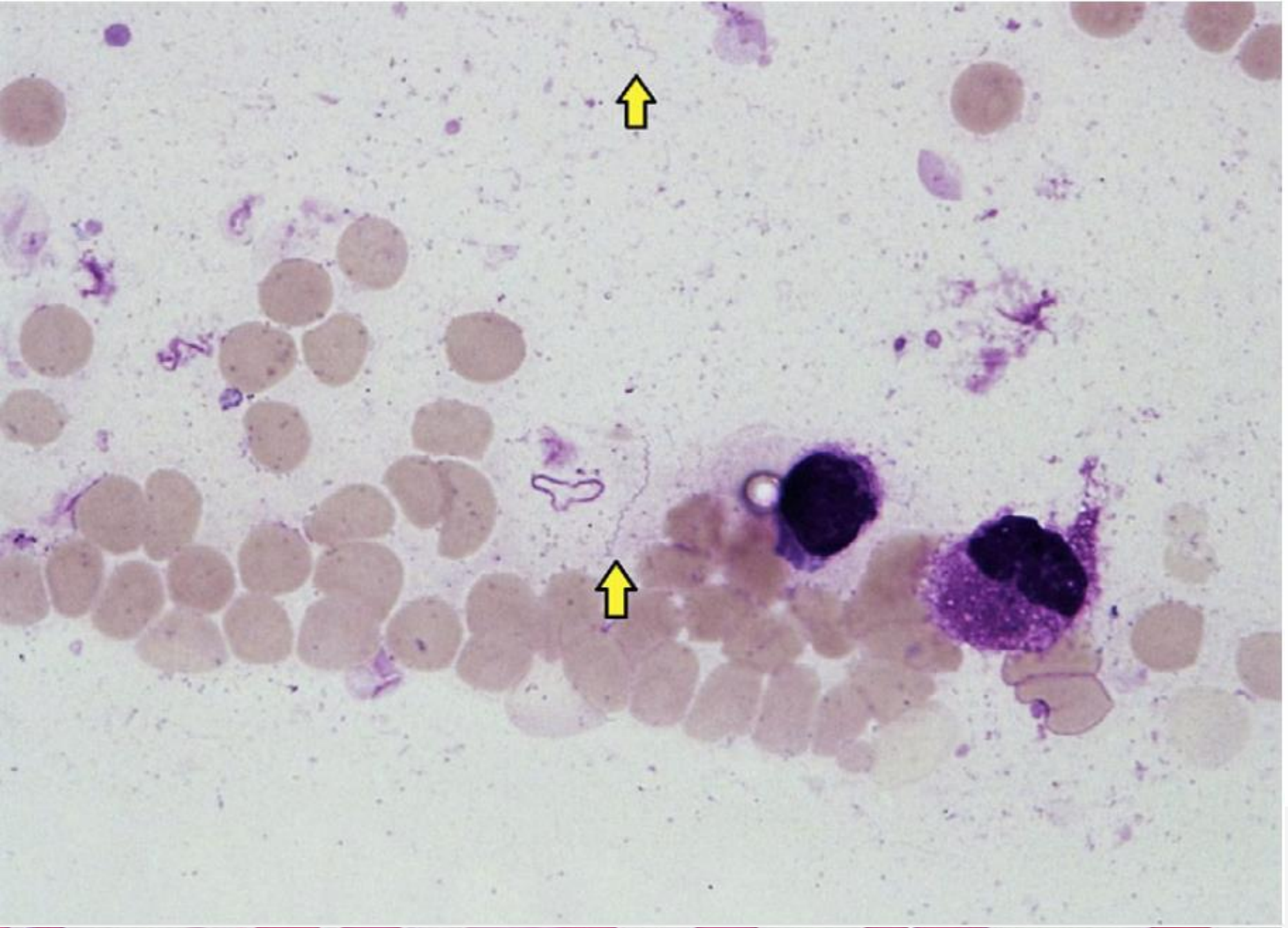
Epidemiol. Infect. (2016), **144**, 189–197. © Cambridge University Press 2015
doi:10.1017/S0950268815000850

Epidemiology of meningitis with a negative CSF Gram stain: under-utilization of available diagnostic tests

L. NESHER[1,2]*, C. M. HADI[3], L. SALAZAR[1], S. H. WOOTTON[4], K. W. GAREY[5,6], T. LASCO[5], A. M. LUCE[7] AND R. HASBUN[1]

Table 3. *Aetiologies of 323 patients with meningitis and a negative cerebrospinal fluid Gram stain*

Aetiology	Method of detection	New Orleans cohort (*N* = 205) *n*/*N* (%)	Houston cohort (*N* = 118) *n*/*N* (%)	All cohorts (*N* = 323) *n*/*N* (%)	*P* value
Unknown		148/205 (72·1%)	63/118 (53·4%)	211/323 (65·3%)	<0·01
Confirmed aetiology		42/205 (20·5%)	47/118 (39·8%)	89/323 (27·5%)	<0·01
Viral		17/205 (8·3%)	31/118 (26·3%)	48/323 (14·9%)	<0·01
Enterovirus	By PCR on CSF	7	6	13	
	By viral culture	1	0	1	
Herpes simplex 1 and 2	PCR on CSF	4	8	12	
Varicella zoster virus	PCR on CSF	3	3	6	
St Louis encephalitis	Serology on CSF or serum	1	6	7	
West Nile virus	Serology on CSF or serum	1	7	8	
Acute HIV	PCR on serum with negative serology	0	1	1	
Bacterial		12/205 (5·8%)	10/118 (8·5%)	22/323 (6·8%)	0·36
Streptococcus pneumoniae	CSF cultures, blood cultures and BinaxNow	3	4	7	
Staphylococcus aureus	CSF cultures and blood cultures	5	2	7	
Group b streptococcus	CSF cultures and blood cultures	1	3	4	
Listeria monocytogenes	CSF culture	1	0	1	
Escherichia coli	blood cultures	2	1	3	
Other		13/205 (6·3%)	6/118 (5·1%)	19/323 (5·9%)	0·8
Cryptococcus neoformans	Cryptococcal antigen on CSF/serum or growth CSF culture	8	2	10	
Treponema pallidum	VDRL on CSF	1	2	3	
Mycobacterium tuberculosis	By CSF cultures or respiratory cultures	2	1	3	
CNS bleed	Imaging	2	0	2	
Histoplasma capsulatum	CSF Fungal cultures	0	1	1	
Presumed aetiology		15/205 (7·3%)	8/118 (6·8%)	23/323 (7·1%)	0·99
Mycoplasma pneumonia	Serology (+IgM and –IgG)	2	2	4	
Rocky mountain spotted fever	Serology (+IgM and –IgG)	3	1	4	
Cytomegalovirus	Serology (+IgM and –IgG)	3	1	4	
Epstein–Barr virus	Serology (+IgM and –IgG)	1	2	3	
Lymphocytic choriomeningitis virus	Serology	2	1	3	
Bartonella henselae	Serology	1	1	2	
Toxoplasma gondii	Positive serology with radiological findings in AIDS patient	1	0	1	
Ehrlichia chaffeensis	By IFA on serum	1	0	1	
CNS vasculitis	Imaging	1	0	1	



SBAC
Sociedade Brasileira de Análises Clínicas

Observational Study

Medicine®

OPEN

Utility of point-of-care Gram stain by physicians for urinary tract infection in children ≤36 months

Toshifumi Yodoshi, MD[a,b,c,*], Masato Matsushima, MD, PhD, MPH[b], Tomohiro Taniguchi, MD[d], Saori Kinjo, MD[a]

Medicine (2019) 98:14(e15101)

Received: 30 November 2018 / Received in final form: 4 March 2019 / Accepted: 11 March 2019

http://dx.doi.org/10.1097/MD.0000000000015101

Table 1

Clinical and laboratory characteristics of this study.

Variable	All subjects, n = 1546
Age; month (IQR)	3 (1, 15)
Female sex; n (%)	696 (45%)
Multiple UTI episodes; n (%)	20 (1.3%)
Positive urine cultures; n (%)	183 (12%)

Table 3

Bacteria isolated from urine cultures.

	Number, percentage (%)	Special bacteria
Gram-negative rod		
E coli	126 (68.5%)	ESBL 12 (9.5%)
Klebsiella spp.	22 (12.0%)	ESBL 2 (9.1%)
Enterobacter spp.	4	
P mirabilis	4	ESBL 2 (50%)
P aeruginosa	1	
Gram-positive coccus		
E faecalis	11 (6.0%)	
S agalactiae	2	
Multiple identified bacteria	8	*E. faecalis* 7 (3.8%)
Others	5	MRSA 1

Table 4

Concordance rate of the point-of-care gram stain and urine culture.

	Urine culture			
	None	GNR	GPC	Multiple-organism
Point-of-care Gram stain				
None	1339	28	4	2
GNR	18	128	1	6
GPC	6	4	10	0
Multiple identified bacteria	0	0	0	0

Table 2

Properties of rapid tests for UTI.

	Sensitivity %, [95%CI]	Specificity %, [95%CI]	PPV %, [95%CI]	NPV %, [95%CI]	Positive LR [95%CI] /Negative LR [95%CI]
Pyuria on UA	73.2% [0.662–0.795]	95.1% [0.938–0.962]	66.7% [0.609–0.720]	96.4% [0.954–0.971]	14.9 [11.6–19.1]/0.28 [0.22–0.36]
Point-of-care Gram stain	81.4% [0.750–0.868]	98.2% [0.974–0.989]	86.1% [0.806–0.903]	97.5% [0.967–0.982]	46.2[30.9–69.2]/0.19[0.14–0.26]
UA (pyuria) and point-of-care Gram stain	70% [0.627–0.765]	99.6% [0.990–0.998]	95.5% [0.905–0.980]	96.1% [0.952–0.969]	158.9[71.1–355.1]/0.30[0.24–0.38]
UA (pyuria) or point-of-care Gram stain	100% [0.980–1.00]	95.8% [0.946–0.968]	76.3% [0.714–0.805]	100%	23.9[18.6–30.8]/0.00

46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

DIAGNOSTIC ACCURACY OF CEREBROSPINAL FLUID GRAM STAIN IN CHILDREN WITH SUSPECTED BACTERIAL MENINGITIS

Kate Brizzi, MD, Elizabeth M. Hines, BA,*†*
*Karin L. Mcgowan, PhD,‡§ and Samir S. Shah, MD, MSCE¶‖***

***Pediac Infec Dis* 2012:31**

TABLE 1. Test Characteristics of Cerebrospinal Fluid Gram Stain

	Definite Meningitis	Definite or Probable Meningitis
All patients	N = 17	N = 21
Sensitivity (%)	94.1 (71.3–99.9)	95.2 (76.2–99.9)
Specificity (%)	99.1 (98.5–99.4)	99.3 (98.8–99.6)
Positive predictive value (%)	47.1 (29.8–64.9)	58.8 (40.7–75.4)
Negative predictive value (%)	99.9 (99.7–100.0)	99.9 (99.7–100.0)
Pretreated with antibiotics	N = 6	N = 9
Sensitivity (%)	100.0 (54.1–100.0)	100.0 (66.4–100.0)
Specificity (%)	72.2 (46.5–90.3)	86.7 (59.5–98.3)
Positive predictive value (%)	54.5 (23.4–83.3)	81.8 (48.2–97.7)
Negative predictive value (%)	100.0 (75.3–100.0)	100.0 (75.3–100.0)
Not pretreated with antibiotics	N = 11	N = 12
Sensitivity (%)	90.9 (58.7–99.8)	91.7 (61.5–99.8)
Specificity (%)	85.2 (66.3–95.8)	88.5 (69.8–97.6)
Positive predictive value (%)	71.4 (41.9–91.6)	78.6 (49.2–95.3)
Negative predictive value (%)	95.8 (78.9–99.9)	95.8 (78.9–99.9)
Age <2 mo	N = 4	N = 6
Sensitivity (%)	100.0 (39.8–100.0)	100.0 (54.1–100.0)
Specificity (%)	99.1 (98.0–99.7)	99.4 (98.4–99.8)
Positive predictive value (%)	40.0 (12.2–73.8)	60.0 (26.2–87.8)
Negative predictive value (%)	100.0 (99.4–100.0)	100.0 (99.4–100.0)
Age ≥2 mo	N = 13	N = 15
Sensitivity (%)	92.3 (64.0–99.8)	93.3 (68.1–99.8)
Specificity (%)	99.1 (98.4–99.5)	99.2 (98.6–99.6)
Positive predictive value (%)	50.0 (29.1–70.9)	58.3 (36.6–77.9)
Negative predictive value (%)	99.9 (99.6–100.0)	99.9 (99.6–100.0)

Values in parentheses represent 95% confidence intervals.

Table 3

Suspected Microorganisms and Accuracy of Gram Stain*

Suspected Microorganism by Gram Stain	Total Episodes	Gram Stain Results Matched Culture Identification	Mismatched Episodes
Gram-positive cocci			
Staphylococcus spp	178 (36.9)	178 (100.0)	0 (0.0)
Streptococcus			
Streptococcus pneumoniae	12 (2.5)	12 (100)	0 (0)
Other streptococci	35 (7.3)	35 (100)	0 (0)
Other gram-positive cocci/undetermined	3 (0.6)	2 (67)	1 (33.3)
Gram-positive rods			
Corynebacterium spp	4 (0.8)	4 (100)	0 (0)
Gram-negative cocci			
Neisseria/Moraxella spp	1 (0.2)	1 (100)	0 (0)
Gram-negative rods			
Enterobacteriaceae family	147 (30.5)	138 (93.9)	9 (6.1)
Pseudomonas spp and related organisms	27 (5.6)	27 (100)	0 (0)
Other gram-negative rods/undetermined	16 (3.3)	16 (100)	0 (0)
Fungi			
Yeasts	21 (4.4)	21 (100)	0 (0)
Multiple microorganisms	38 (7.9)	36 (95)	2 (5)
Total	482 (100.0)	470 (97.5)	12 (2.5)

* Data are given as number (percentage).

Eur J Clin Microbiol Infect Dis (2007) 26:325–329
DOI 10.1007/s10096-007-0291-7

ARTICLE

Differentiation of *Candida albicans* from non-*albicans* yeast directly from blood cultures by Gram stain morphology

A. Harrington · K. McCourtney · D. Nowowiejski · A. Limaye

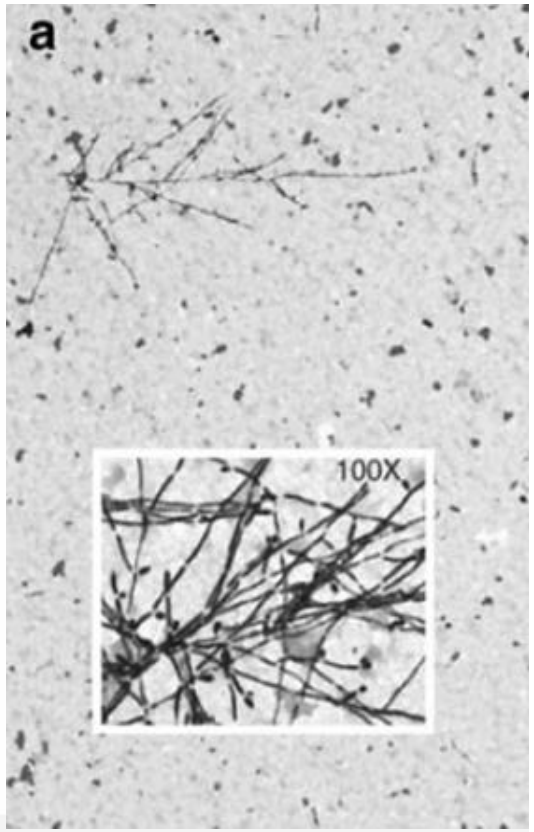


Table 2 Presence or absence of pseudohyphae clusters according to the results of culture and the type of blood culture bottle

	C. albicans	Non-*C. albicans*
First positive blood culture		
Pseudohyphae clusters present	22	1
No pseudohyphae clusters	4	33
Aerobic bottle		
Pseudohyphae clusters present	24	1
No pseudohyphae clusters	1	19
Myco/F Bottle		
Pseudohyphae clusters present	2	0
No pseudohyphae clusters	6	8

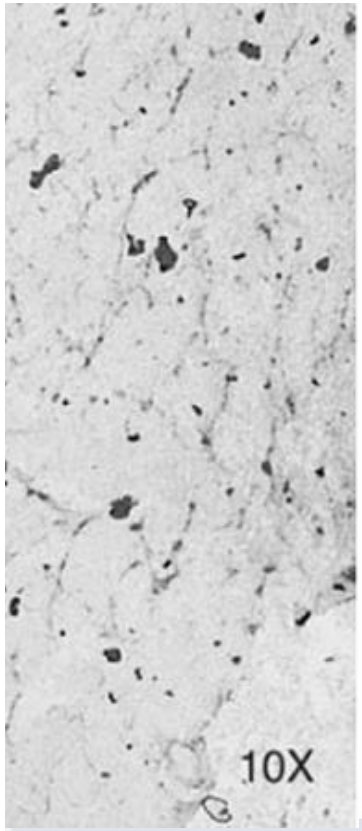


Table 1. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) findings in positive blood culture bottles by group with a cut-off of ≥1.7 and with no cut-off

Group	Cut-off ≥2.0			Cut-off ≥1.7			No cut-off			
	Correct ID	MisID	No peaks/ score <2.0	Correct ID	MisID	No peaks/ score <1.7	Correct ID	MisID	MisID + microscopy[1]	No peaks
Enterobacteriaceae n = 151 (Gram-negative rods)										
Escherichia coli	85	0	17	92	0	10	93	0	0	9
Enterobacter spp.	3	1	3	4	1	2	6	1	1	0
Citrobacter spp.	5	0	2	7	0	0	7	0	0	0
Klebsiella spp.	19	1	4	20	1	3	21	1	1	2
Serratia spp.	5	0	2	7	0	0	7	0	0	0
Others	3	0	1	3	0	1	3	1	0	0
Streptococcus spp. n = 98 (Gram-positive cocci in chains/diplococci)										
Streptococcus pneumoniae	1	0	59	4	0	56	15	21	0	24
Haemolytic group A,B,C,G	0	0	9	0	0	9	1	5	0	3
Viridans group streptococci	0	0	29	0	0	29	4	20	1	5
Enterococcus n = 33 (Gram-positive cocci in chains/diplococci)										
Enterococcus faecium	2	0	16	5	0	13	10	7	0	1
Enterococcus faecalis	0	0	15	1	0	14	7	6	1	2
Staphylococcus and *Micrococcus* n = 185 (Gram-positive cocci in clusters)										
Staphylococcus aureus	0	0	64	4	0	64	23	34	2	11
Staphylococcus lugdunensis	1	0	1	1	0	1	2	0	0	0
Coagulase negative Staphylococci (CoNS)	15	0	96	39	0	72	68	35	1	8
Micrococcus spp.	2	0	2	4	0	0	4	0	0	0
Aerobic/NF Gram-negative rods n = 18 (Gram-negative rods)										
Pseudomonas aeruginosa	12	0	2	12	0	2	12	0	0	2
Others	2	0	2	3	0	1	3	0	0	1
Miscellaneous n = 56 (Yeast, Gram-negative rods, Gram-negative diplococci, Gram-positive rods)										
Yeast	0	0	19	0	0	19	1	18	1	0
Anaerobic	2	0	5	3	0	4	4	1	1	2
Others	2	0	28	5	0	25	10	16	3	4
Mixed cultures[2] n = 37	8	0	29	15	0	22	25	8	0	4
Culture negative n = 5	0	0	5	0	0	5	0	5	0	0

[1]Misidentifications when combining MALDI-TOF-MS and microscopy findings.
[2]A correct identification of one pathogen in the mixed infection.

Is the Gram Stain Useful in the Microbiologic Diagnosis of VAP? A Meta-analysis

John C. O'Horo,[1] Deb Thompson,[2] and Nasia Safdar[3,4]

[1]Department of Graduate Medical Education, Aurora Healthcare Metro, [2]Infectious Disease Associates, Milwaukee; [3]William S. Middleton Memorial Veterans Hospital, and [4]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison

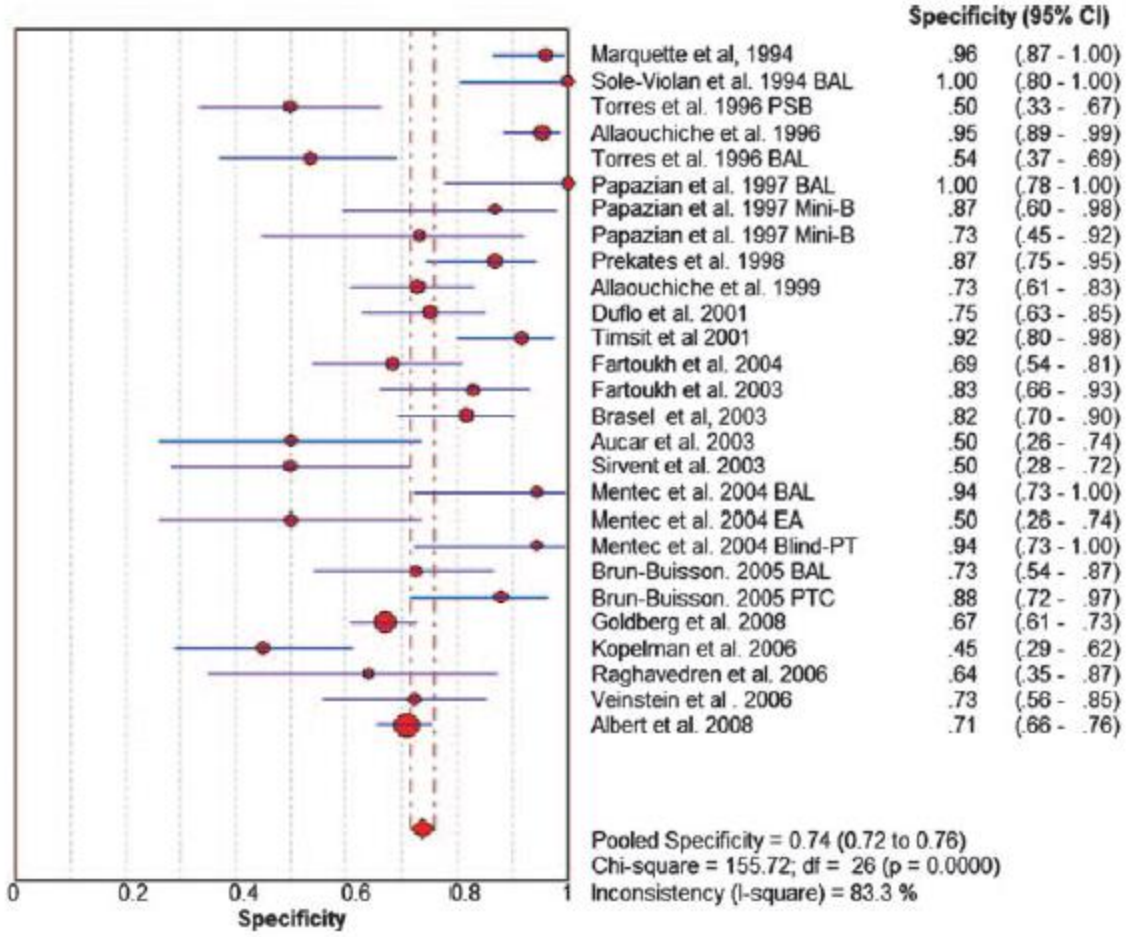


Figure 3. Pooled specificity of Gram stain for diagnosis of VAP. Abbreviations: BAL, bronchoalveolar lavage; Blind-PT, blind plugged telescoping catheter; CI, confidence interval; EA, endotracheal aspirate; Mini-B, mini bronchoalveolar lavage; PSB, protected specimen brush; PTC, plugged telescoping catheter; VAP, ventilator-associated pneumonia.

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1991, p. 297–301
0095-1137/91/020297-05$02.00/0

Vol. 29, No. 2

46º Congresso Brasileiro de Análises Clínicas
7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

Reliability of Diagnosing Bacterial Vaginosis Is Improved by a Standardized Method of Gram Stain Interpretation

ROBERT P. NUGENT,[1]* MARIJANE A. KROHN,[2] AND SHARON L. HILLIER[3]

Pediatric, Adolescent, and Maternal AIDS Branch, National Institute of Child Health and Human Development, Executive Plaza North, Bethesda, Maryland 20892,[1] and Department of Epidemiology[2] and Department of Obstetrics and Gynecology,[3] University of Washington, Seattle, Washington 98195

Received 27 April 1990/Accepted 22 September 1990

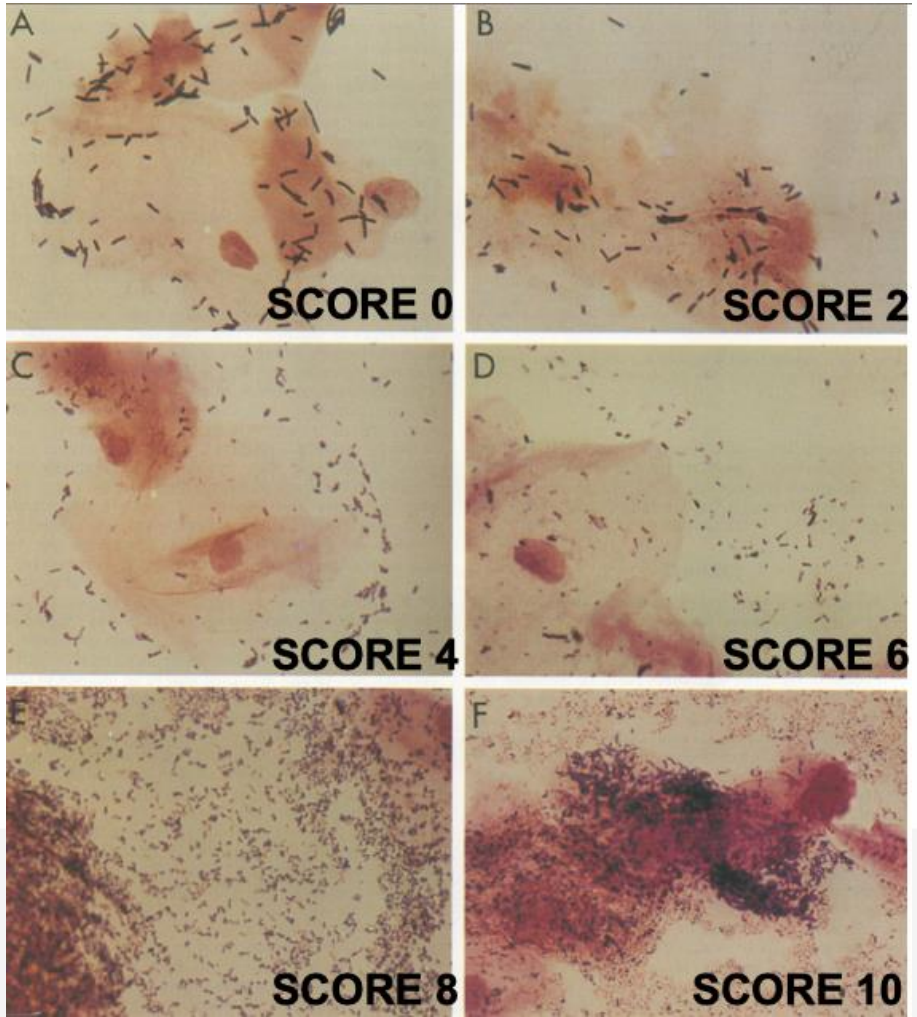
Laboratory examination of vaginal smears and the determination of the Nugent Score

N Score = The sum of the scores for each bacterial morphotype listed below. (Note Number of Organisms seen / 100X objective)

Lactobacilli	SCORE	*Gardnerella, Bacteroides*	SCORE	Curved gram-negative bacilli	SCORE	Sum=*N-SCORE
30 or >	0	0	0	0	0	0
5-30	1	<1	1	<1	1	3
1-4	2	1-4	2	1-4	1	5
<1	3	5-30	3	5-30	2	8
0	4	30 or >	4	30 or >	2	10

***Interpretation of Nugent Score**

If N Score is:	AND:	Then Report:
0-3		Smear NOT consistent with BV
4-6	Clue Cells **NOT** present	
4-6	Clue Cells **ARE** present	Smear consistent with BV
≥ 7		



ORIGINAL ARTICLE

Sensitivity of Gram stain in the diagnosis of urethritis in men

Mª Angeles Orellana,[1] Mª Luisa Gómez-Lus,[2] David Lora[3]

Table 2 Sensitivity, specificity and LR (+) of Gram stain to urethritis, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Ureaplasma urealyticum*

	Gram stain	Reference method		Sensitivity % (95% CI)	Specificity % (95% CI)	LR (+) (95% CI)
N=491		+	−			
Urethritis >2 PMNLs/HPF	+	56	71	38 (30 to 46)	79 (75 to 84)	1.8 (1.4 to 2.4)
	−	92	272			
Urethritis >5 PMNLs/HPF	+	38	32	26 (18 to 33)	91 (87 to 94)	2.7 (1.8 to 4.2)
	−	110	311			
Neisseria gonorrhoeae >5 PMNLs/HPF	+	24	46	80 (64 to 96)		
	−	6	415			
Chlamydia trachomatis >5 PMNLs/HPF	+	8	62	23 (8 to 39)		
	−	26	395			
Ureaplasma urealyticum >5 PMNLs/HPF	+	6	64	11 (2 to 20)		
	−	49	372			

PMNLs/HPF, polymorphonuclear leucocytes/high-power field.
Reference methods are described in items 2, 3 and 4 of Materials and methods.

Sex Trans Infec 2012:88

46º Congresso Brasileiro de Análises Clínicas

7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

AUTOMAÇÃO

- ✓ Sistema prático e compacto
- ✓ Padronização
- ✓ Rapidez
- ✓ Sistema ecológico
- ✓ Rastreabilidade
- ✓ Sem contaminação cruzada
- ✓ Touchscreen
- ✓ Opção de citocentrífuga

PREVI® Color Gram

BIOMÉRIEUX

SBAC
Sociedade Brasileira de Análises Clínicas

SUGESTÕES

- ✓ Usar sempre lâminas novas
- ✓ Avaliar qualidade da coloração visualizando o núceo dos leucócitos
- ✓ Realizar Gram de toda amostra analisada (exceto urina*)
- ✓ Substituir a fucsina por safranina
- ✓ Filtrar os corantes regularmente*
- ✓ Sempre fazer correlação clínica
- ✓ Fazer Gram da gota de urina não centrifugada
- ✓ Sempre ter pelo menos uma lâmina de reserva
- ✓ Sempre reportar: ”Aguarda cultura para confirmação”.

46º Congresso Brasileiro de Análises Clínicas
7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

LAUDOS

- ✓ **Reportar a morfologia colonial básica de forma quantitativa e, se possível, fazer uma associação com possíveis microrganismos**
  - ✓ **Numerosos cocos Gram positivos em cadeias compatíveis com estafilococos.**
  - ✓ **Alguns bacilos Gram negativos curvos sugestivos de *Campylobacter* spp.**
- ✓ **Reportar resposta inflamatória de forma quantitativa e o mais específica possível**
  - ✓ **Numerosos polimorfonucleares.**
- ✓ **Reportar células epiteliais, quando justificado**
  - ✓ **Numerosas células epiteliais escamosas, sugestivas de contaminação vaginal. Sugerimos, a critério clínico, repetição de coleta.**
  - ✓ **Várias ”Clue-Cells”.**
- ✓ **Reportar padrões microscópicos que possam orientar o diagnóstico**
  - ✓ **Diplococos Gram negativos intracelulares sugestivos de *Neisseria gonorrhoeae**.**
  - ✓ **Cocobacilos pleomórifcos Gram variáveis supracitoplasmáticos semelhantes à *Gardnerella vaginalis*.**
- ✓ **Reportar outras estruturas que possam agregar valor diagnóstico**
  - ✓ **Alguns espermatozoides.**

SBAC
Sociedade Brasileira de Análises Clínicas

46º Congresso Brasileiro de Análises Clínicas
7º NÚCLEO DE GESTÃO E QUALIDADE | 5º FÓRUM DE PROPRIETÁRIOS DE LABORATÓRIOS

CONCLUSÕES

- ✓ O Gram é uma metodologia com excelente custo benefício e que agrega valor ao laudo de microbiologia clínica.
- ✓ Como qualquer metodologia necessita padronização (microscopia) e constante controle de qualidade.
- ✓ Vários padrões microscópicos estão muito bem estabelecidos com patologias.
- ✓ Fundamental para dar uma informação preliminar ao clínico de boa qualidade, facilitando o diagnóstico e manejo terapêutico.
- ✓ Cabe ao microbiologista responsável avaliar, de acordo com as características do seu laboratório, a melhor maneira de utilizar essa ferramenta em seu serviço.

SBAC
Sociedade Brasileira de Análises Clínicas

Promoção e realização

SBAC
Sociedade Brasileira de Análises Clínicas

Alessandro C. O. Silveira
alessandro.silveira@dasa.com.br
(47) 99974-1213