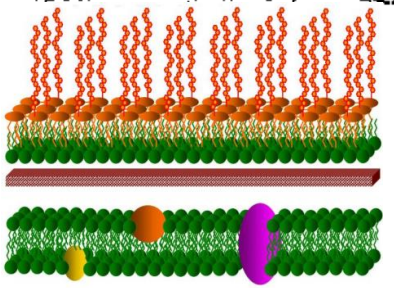
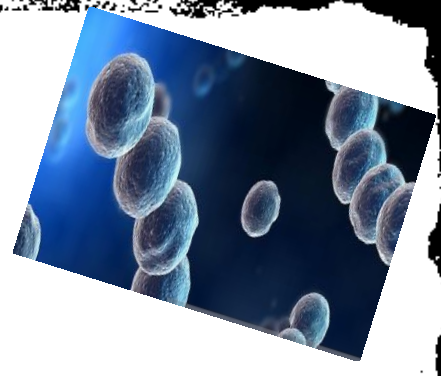




Gram stain (past, present and future)

Nadia Makki

Microbiology /Imelda

Dr. Johan Frans

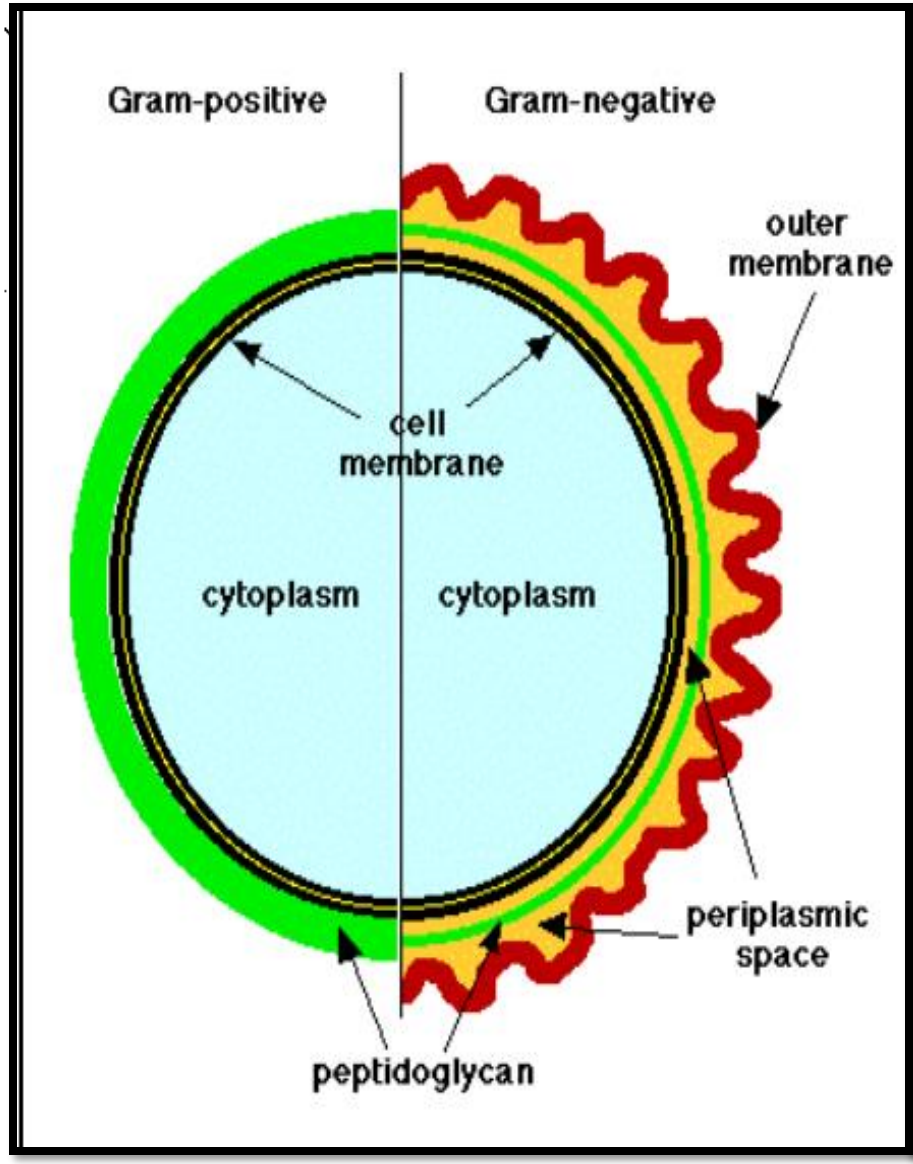
Dr. Annick Simsmans

Dr. Erwin HO

2 Cases:

- **Case 1/** Female, 42 y, breast CA.
  - Positive blood culture →Gram stain.
  - **Yeast and *Staphylococcus spp***. were seen on the gram stained smear.
  - Empirical therapy was directly adjusted

- **Case 2/** Male, 66 y, alcoholic,
  - Presented with pneumonia and epileptic attack.
  - Ceftriaxone was started (P.S. amoxi-clav from the GP).
  - CSF Gram stain and culture (48 h incubation) were negative.
  - Multiplex PCR was positive for ***Listeria monocytogenes***.
  - Antibiotic therapy was adjusted by adding amoxicillin and gentamicin.



Hans Christian Joachim Gram (1853-1938)

The situation after more than a century:

- Gram stain is part of the standard protocol of many clinical specimens.
- Gram stain is performed on **direct smears** of primary clinical specimens , or on **indirect smears** from a growth medium.

- Total number **direct** Gram stained smears in Imelda labo 2018:
  - **7.351**
  - Time cost (+/- 45 minutes per day) → **270 hours per year**.

1) What is the clinical impact of direct Gram stain on a clinical specimen?

2) What is the clinical impact of indirect Gram stain on a subculture of a clinical specimen?

What have we done?

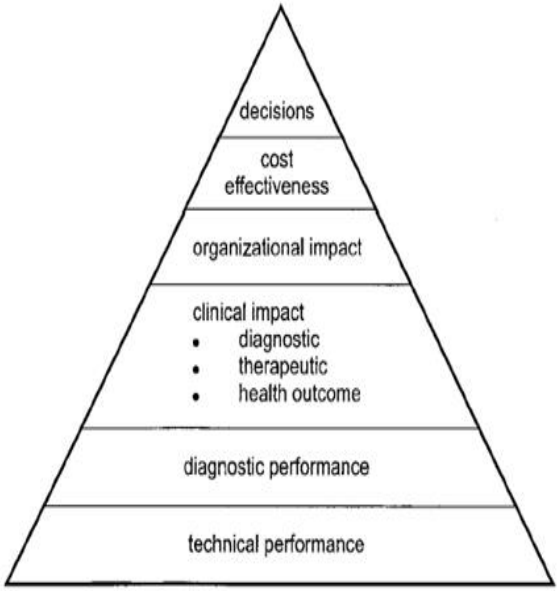
We have discussed direct smears and subcultures apart (**literature/guidelines**):

- Analytical
- Diagnostic
- Clinical impact
- Organizational impact
- Financial impact

- We have sent a **questionnaire** to 7 clinical laboratories.
- Specimen **smears** with relatively high counts of bacteria for gram stain analysis were submitted to 5 of the 7 laboratories.

To keep up ....

Direct Gram stain **Indirect Gram stain**



1.Pre-analytical

1.1.Patient related:
- 1.1.1.Prior use of antibiotic
- 1.1.2.Time to collect specimen

1.2. Sample related:
- 1.2.1.Inappropriate specimen sampling
- 1.2.2 Incorrect transport
- 1.2.3.Delayed transport
- 1.2.4 Sample contamination

1.3.Processing related: centrifugation, smear preparation, staining

2.Analytical

2.1.Detection limit
2.2.Accuracy
2.3.Correlation
2.4.Precision

3. Quality factors

3.1.External quality control
3.2. Internal quality control
3.3. The competency testing

4.Diagnostic performance

5.Clinical impact

6.Organazational impact

7.Financial impact

1.Pre-analytical:

1.1.Patient related:

1.1.1. Prior use of antibiotic

1.1.2.Time of specimens collection

1.2.Sample related:

1.2.1.Inappropriate specimen sampling

1.2.2.Effect transport medium

1.2.3.Delayed transport

1.2.4.Sample contamination

1.3.Processing related:

1.3.1.Temperature

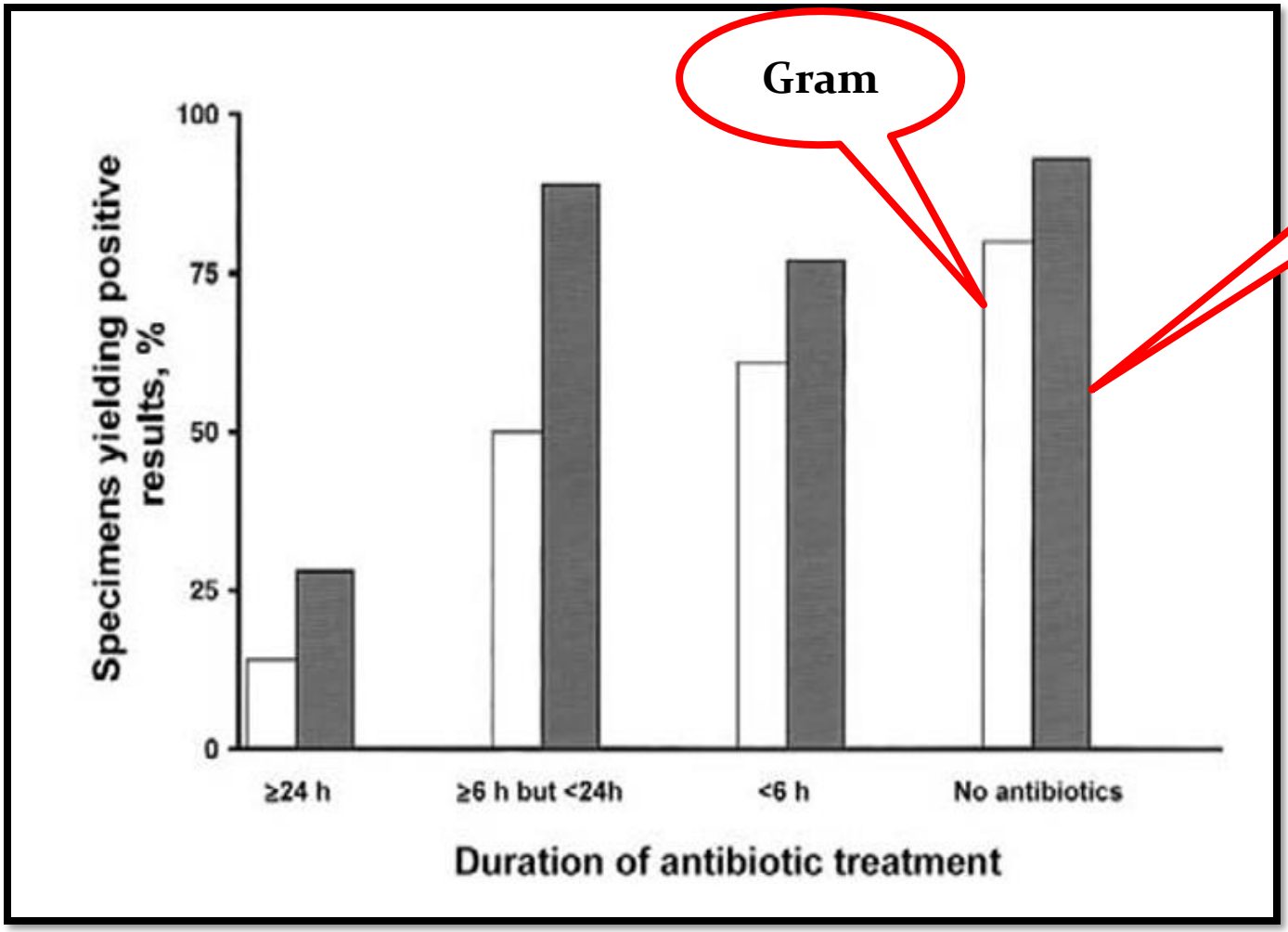
1.3.2.Centrifugation

1.3.3.Smear preparation

1.3.4.Diversity in Gram staining

Effect of prior use of AB on sputum Gram stain:

- Mucher DM et al, 2004 (105 pts. with **pneumococcal pneumonia**):



Gram staining *(open bars)* and culture *(shaded bars)* for detection of *S.pneumoniae* in patients with proven pneumococcal pneumonia (Mucher DM et al Clin Infect Dis .2004.)

Effect of prior use of AB on CSF Gram stain:

- Bohr V [1]. et al:
  - Pre admission treatment with **antibiotic may hinder but not prevent** the bacteriological diagnosis of meningitis
  - The diagnosis of meningococcal meningitis was mostly affected.

- Greenle [2] et al.:
  - Sensitivity in pt. with meningitis is 60%–80% (without AB), much lower **40%–60% (with AB).**

- Nigrovic Le [3]/ Blazer S.[4] et al.**:**
  - CSF **cellularity** and PMN are **not significantly altered** after AB.

1 Bohr V. et al. J Infect. 1983
2 Greenlee JE et al. Infect Dis Clin North Am. 1990
3 Lise E. Nigrovic et al.. Pediatrics Oct 2008
4 Blazer S. et al. Am J Clin Pathol. 1983

Effect of prior use of AB on pleural fluid Gram stain:

- Becker [1] et al, 2011 :
  - A prospective study including 110 children with parapneumonic effusion.
  - 50% had received **antibiotics at least 48 hours** before pleural fluid analysis.
  - It has a **negative impact on the identification of bacteria** by Gram (<0,027).
  - It did **not interfere significantly with biochemical parameters** of pleural fluid (pH, glucose, and LDH).

- No other studies available

- Samples affected → CSF, pleural fluid, sputum.
- No data available → other samples

1 Becker et al. J Pediatr Surg. 2011

1.Pre-analytical:

1.1.Patient related:

1.1.1. **Prior use of antibiotic**

1.1.2.Time of specimens collection

1.2.Sample related:

1.2.1.Inappropriate specimen sampling

1.2.2.**Effect transport medium**

1.2.3.Delayed transport

1.2.4.Sample contamination

1.3.Processing related:

1.3.1.Temperature

1.3.2.Centrifugation

1.3.3.Smear preparation

1.3.4.**Diversity in Gram staining**

Effect of transport medium :

- **Fontana C.**[1] et al, 2009:
  - Quality of smear from the ESwab (using 100 µl of Amies medium) was superior to those obtained using the Amies gel Transystem

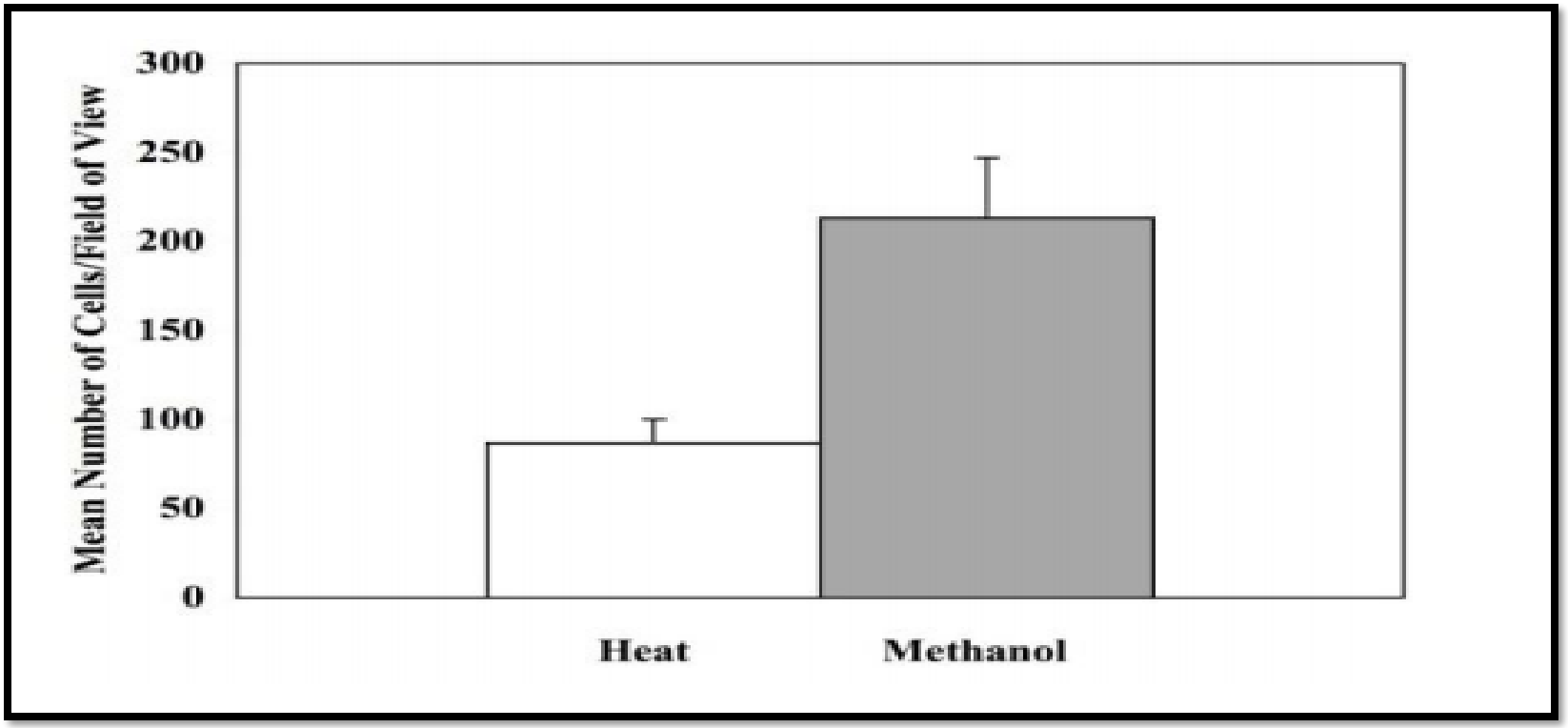
Specimen types	Results are expressed as: no. of slides presenting differences in microscopic observation of human cells and/or of microbial elements/no. of samples tested		
(no.)	ESwab Volumes for slides preparation		
	100 µl§	50 µl§	Amies Gel slides
Vaginal Swab (32)	32/32	26/32	16/32
Cervical Swab (27)	27/27	25/27	15/27
Urethral Swab (11)	11/11	11/11	8/11
Wound Swab (10)	10/10	10/10	7/10
Total (80)	80/80 (100%)	72/80(90%)	46/80 (57.5%)
P value		P = 0.16	P = 0.04

§ = the results were the same even after 24 and 72 h storage.

1 Fontana C. et al. . BMC Res Notes. 2009

Effect of sample processing (staining):

- **Jeanne M. M.**[1] et al/**Mangels JI** [2] et al./ **Magee CM** [3] et al.:
  - Methanol-fixed gram-positive bacterial cells were **less sensitive to decolorization** during the Gram staining procedure than were heat-fixed cells



1 Jeanne M.M. et al. . *J Biol. Teach*. 2009
2 Mangels JI et al. Diagn Microbiol Infect Dis. 1984
3 Magee CM et al. Am J Surg. 1975

2.Analytical:

2.1. Detection limit : 10^4 to 10^5 organisms/ml

2.2. Accuracy

2.3. Correlation with culture

2.4. Precision

2.Analytical:

2.2. Accuracy:

- Samuel LP [1] et al, 2016:
  - Misinterpretation was mostly with mixed infection or GPC.

Site	No. (%) of reader errors
A	6/67 (9)
B	24/78 (31)
C	12/74 (16)
D	20/44 (45)
Total	62/263 (24)

Reader error
(sputum, biopsies, wounds)

- Q-Probes study [2] (**positive blood cultures**):
  - Median discrepancy rate (**1%**)
  - Highest discrepancy rate (**20.8%**) **for mixed cultures**

1 Samuel LP. et al. Clin Microbiol.2016
2 Schifman RB. et al. Arch Pathol Lab Med. 2015

2.Analytical:

2.3. Correlation with culture:

- Samuel LP [1] et al:
  - **High correlation** rate 94% **cultures with** (3+, 4+) colonies
  - Less correlation (76%) with culture (2+) colonies
  - The lowest correlation (29%) cultures with (1+) colonies

1 Samuel LP. et al. Clin Microbiol.2016

2.Analytical:

2.4. Precision:

- **Precision (Bartlett RC [1] et al.):**

- Preparation of suspensions of cells and bacteria that yielded identical smears.

- Concordance of technologists' observations :

  Bacterial identification category **: 100%**

  Bacterial enumeration: 45-96%

  Neutrophils : 72-78%

  Squamous cells: 68-78%

1 Bartlett RC et al. Am J Clin Pathol. 1979

2.Analytical (precision): results Gram stain analysis from 6 micro. laboratories.

Slide	Sample type	Gram stain (Lab 1)	Gram stain (Lab 2)	Gram stain (Lab 3)	Gram stain (Lab4)	Gram stain (Lab 5)	Gram stain (Lab 6)	Culture
1	**Perianal abscess**	WBC+++ GNR+++ GPC rare	WBC ++ RBC ++ mixed flora +++	WBC+++ RBC++ GNR+++ GPC+++	WBC ++ RBC + GNR++ GPC +	WBC +++ RBC ++ GNR+++ GPC++	WBC+++ RBC+++ GNR+++ GPC +	**S.anginosus+++** **S. agalactiae++** **C. freundii (enrichment)** **H.parainfluenzae afew** **B. fragilis+++**
2	PLEC ++ WBC++ Mixed flora +++ GNR rare GPC ++ **pneumococci?**	PLEC ++ WBC ++ Mixed flora ++	PLEC- WBC- GNR++ GPC+++	 GNR++	PLEC >10 WBC >100/field **(not representatie for deep airways)**	PLEC ++ WBC+++ Mixed flora +++	PLEC ++ WBC++ Mixed flora +++ GNR rare GPC ++ **pneumococci?**	**Mixed flora ++** **P.aerogenosa++** **Yeast a few**
3	**Sputum**	No PLEC WBC +++ GPC +++ **Pneumococci?** Mixed flora +++	PLEC rare WBC +++ GPC +	PLEC+	PLEC rare **cells+ ?**		PLEC + C+++ +++	**S. pneumoniae +++** **Mixed flora ++** **H.parainfluenzae +**
4	**Blood culture**	GPR	GPR or GNR (repeat it again)	GPR and GNR or Gram variable?	GPR and GNR? (repeat it again)	GPR	GNR	**Clostridium ramosum**
5	**Blood culture**	Streptococcus	Streptococcus	Streptococcus	Streptococcus	Streptococcus	Streptococcus	**E. faecalis**
6	**Deep wound (swab) :stoma**	GNR+ GPR+	WBC ++ Mixed flora ++	WBC+++ Mixed flora+++ **(anaerob. ?)**	WBC + RBC+ GNR + GPR + GPC +	WBC ++ RBC ++ GNR+++ GPR ++ GPC++	WBC +++ RBC +++ GNR + GPR rare GPC +	**P. mirabilis (enrichment)** **E. coli+++** **S.vestibularis +++** **S.lutetiensis +++** **E. faecium +** **S.anginosus +** **P.pentosaceus +** **C.perfringens ++**

Sputum: rejected by 1 laboratory

Sputum: suggestive ID not mentioned by 5/6 laboratories

2.Analytical (precision): results Gram stain analysis from 6 micro. laboratories

Slide	Sample type	Gram stain (Lab 1)	Gram stain (Lab 2)	Gram stain (Lab 3)	Gram stain (Lab4)	Gram stain (Lab 5)	Gram stain (Lab 6)	Culture
7	Biopsy (bilioma)	GNR GPR **GPC (Staph.)**	WBC ? Mixed flora +++	WBC++ GNR+++ GPC+ GPR++	WBC+- GNR +++, GPC+ GPR+	No WBCs GNR +++ GPC ++ GPR ++	GNR +++ GPC + GPR +	**C.freundii** **L.johnsonii E. faecium E. faecalis** **B.fragilis** **P.denticola** **C. tropicalis (enrichment)**
8	Vaginal swab	PLEC+++ Clue cells +++ Gram var. rods +++ GPC rare WBC rare	PLEC +++ Clue cells ++ Gramvariale rods +++ Mixed flora+ WBC rare		Gram… rods +++ GPC+ lactobacillus+	PLEC++ Clue cells + **Nugent score: 8** suggestive for bacterial vaginosis.	Microscopic: BV Clue cells 1+	**Gardnerella vaginalis +++** **Normal vaginal flora+** **K. pneumoniae ++**
9	Ear discharge (swab)	WBC ++ GNR +++	PLEC + WBC ++ Mixed flora rare **yeast +**	WBC+ GNR+++	WBC rare GNR…	WBC + GNR…	WBC + GNR +++	**P.aeruginosa +++** **S.epidermidis (enrichment)**
10	Sputum	PLEC +++ WBC+ Mixed flora +++ **Yeast, pseudomyce lim +++**	PLEC ++ WBC ++ Mixed flora +++ Yeast rare	PLEC + No WBC GND++ GPR+ GPC+		…not representati ve for deep airways)	PLEC +++ WBC +++ Mixed flora +++	**Mixed flora +++** **Yeast ++**
11	Jackson -Pratt drain	WBC+++ No bacteria	WBC rare No bacteria RBC +++	PMN+++ No bacteria	broken	WBC + No bacteria RBC +++	WBC + No bacteria RBC +++	**Negative**

Genital samples: lack of standardization in reporting

All samples labo 6: quantitative analysis !!

3.Quality factors:

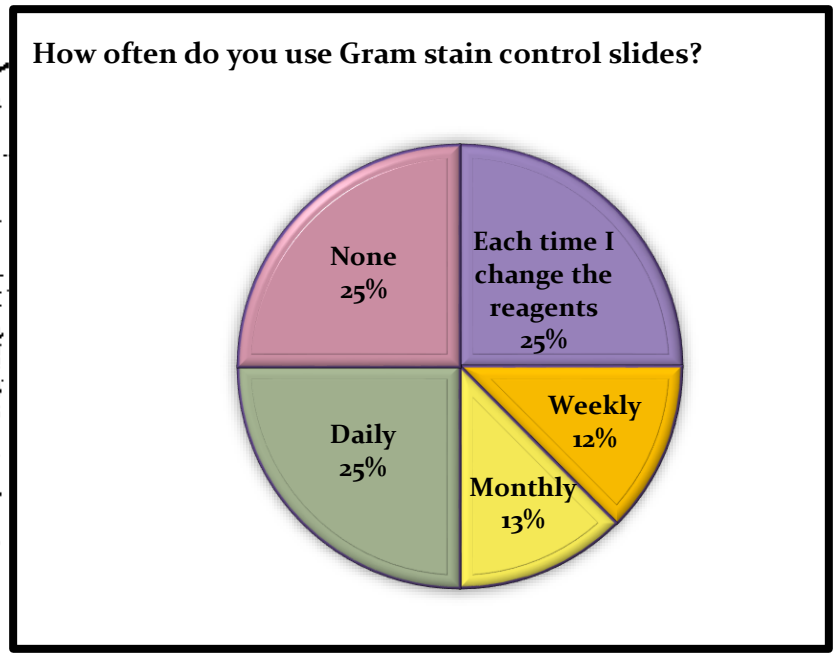
3.1.Internal quality control

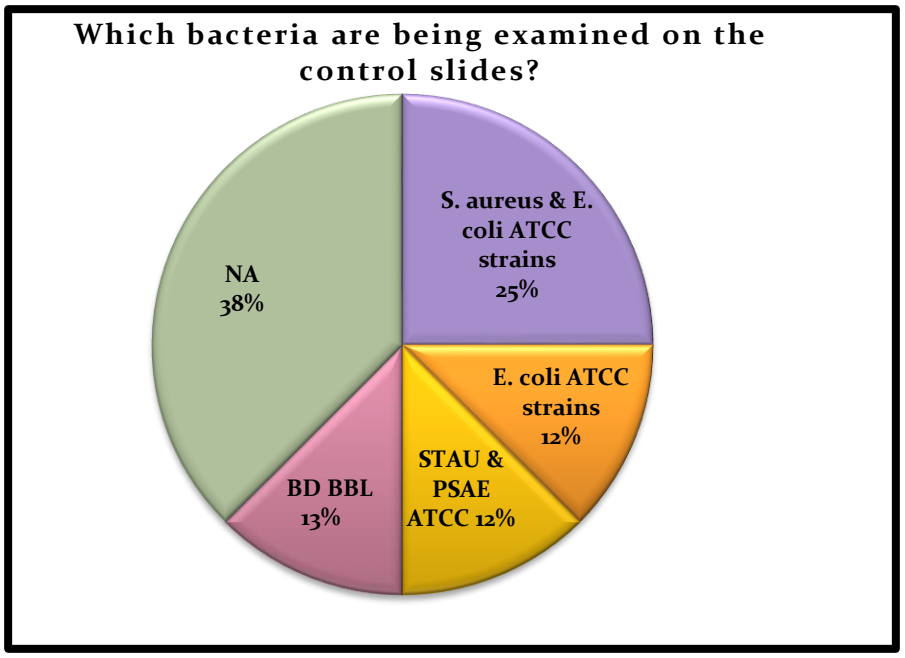
3.2.External quality control

3.3.Competence testing

3.1. Internal quality control

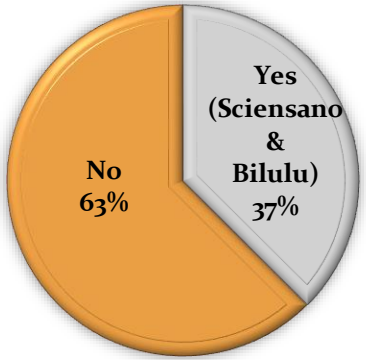
- **Leber 2016/ CAP/ ISO 15189:**
  - Gram stain reagents should be tested with control organisms (known gram-positive and gram negative), with each batch of reagents, lot number and shipment and **weekly** thereafter.





3.2. External quality control

Are you a participant in an external quality assessment program for Gram stain analysis and interpretation?



Sciensano (WIV) EQC, BILULU are actually for identification and susceptibility testing of bacteria, and not for Gram stain.

413	Bacteriology – Gram Stain	Suerbaum / Ziesing
3 slides (heat-fixed)		38,00€ / Survey 19,00€ additional sample
Evaluation of morphology and staining characteristics Participants of the EQA schemes in bacteriology No. 411 or 412 will get a certificate for gram staining if they are successful in this part.		participation according to Rili-BÄK: half-yearly
	Mar. (2)	Oct. (6)
Deadline for registration	01.02.19	30.08.19
Shipment	13.03.19	09.10.19
Deadline for receipt of data	22.03.19	18.10.19

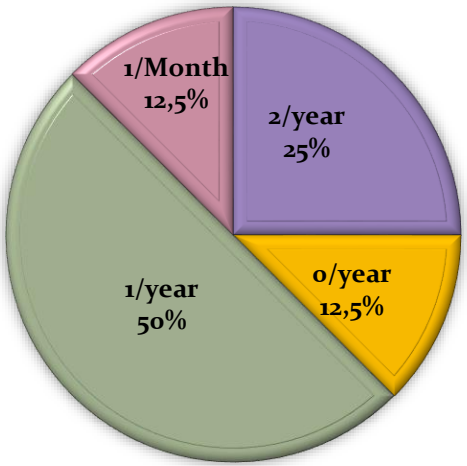
EQC specific for gram stain from INSTAND society (2/year).

- **Q-probes**[1]**:**
  - **96%** monitored **accuracy** of blood culture Gram stains as a quality indicator.
  - **59%** monitored **TAT** blood culture Gram stains (median 45 min.)

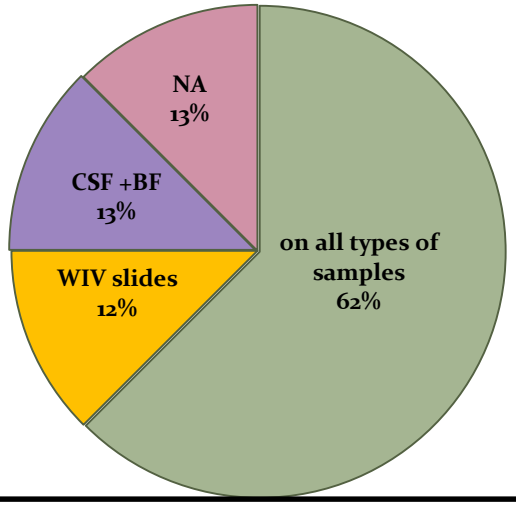
1 Schifman RB. et al. Arch Pathol Lab Med. 2015

3.3. Competence testing:

Competence testing (inter-individual testing) of Gram stain is done:



Competence testing (inter-individual testing) of Gram stain is done:



4. Diagnostic performance of direct Gram stain:

4.1. Respiratory samples:

- O'Horo JC [1] et al, 2012 (**BAL and EA** for VAP):
  - **Sensitivity**: **79%**
  - Specificity: 75%.
  - **NPV: 91%**
  - PPV: 40%.
  - Kappa correlation with culture: **0.42** for Gram pos.
  - Kappa correlation with culture: 0.34 for Gram neg.

- Seligman R [2] et al.:
  - NPV for **EA** was **(92.8%)** for S. aureus in VAP.

- Ref.Gottesman T [3] et al **:**
  - **NPV GPC(97%)** and NPV GNR **(20%).**

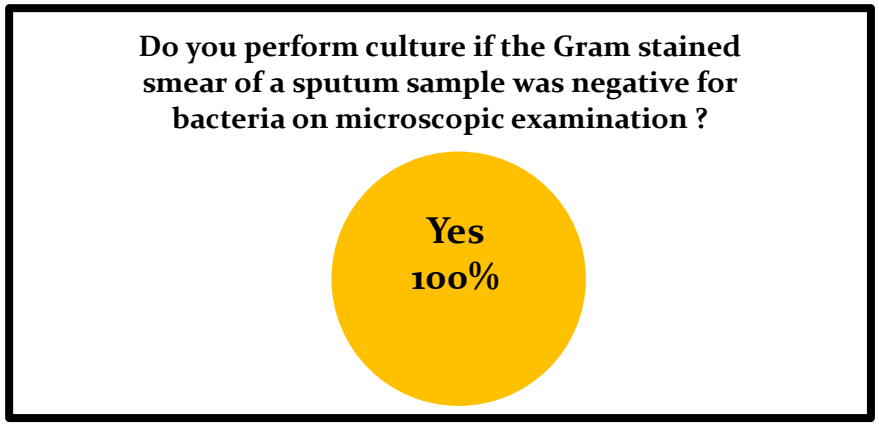
1 O'Horo JC et al. Clin Infect Dis. 2012
2 Seligman R et al. BMC Anesthesiol. 2015
3 Ref Gottesman T. et al. . J Crit Care.2014

4.1. Respiratory samples :

- Musher DM [1] et al, 2004 (**sputum**):
  - Sensitivity: **31%** without exclusion of inadequate samples.
  - Sensitivity: **57%** after exclusion of inadequate samples

- Leber 2016/Nair B [2] et al. (**cystic fibrosis**):
  - Gram stain for sputum of cystic should be only performed **if explicitly requested.**
  - The **rejection criteria should not be applied** on these patients samples.
  - 40% of these samples would be rejected according to the criteria.
  - >90% of the cultures will grow potential pathogens.

- Leber 2016:
  - Cultures should not be performed if the sputum Gram stain was negative for bacteria**.**



1 Mucher DM et al Clin Infect Dis.2004
2 Nair B et al. J Clin Microbiol 2002

Clinical impact:

- **IDSA 2018:** recommended for screening for acceptance of sputum samples.
- A **smear lacking inflammatory cells and a culture negative** for pathogens have a very **high negative predictive value**.

- American and British thoracic society **2007-2009** and European respiratory society **2011**:
  - The **empirical treatment** of hospitalized CAP **should always cover *S. pneumoniae***.
  - It should also cover the atypical causative agents by severe and very severe pneumonia.
  - The treatment should also be directed against *S. aureus* during epidemics of influenza.

- No clinician will narrow the empirical antibiotic therapy for a patient with established severe pneumonia based on Gram results [1].

1 O'Horo JC et al. Clin Infect Dis. 2012

4.2.a Genital samples (vaginal samples for Bacterial vaginosis):

- Diagnosis of BV: presence **Amsel criteria** or **Nugent score**:
  - **Amsel criteria: 3/4** should be present
  - **Sensitivity 70%** (lower in pregnant 62%)
  - Specificity 94%

  } compared to Gram

- Schwebke JR[1] et al.(multicenter study):
  - **Gram stain** gold standard for Dx BV
  - **Sensitivity: 89%**
  - Specificity: 83%

  } compared with Amsel criteria

Amsel criteria:
- Thin, grayish-white **discharge.**
- Vaginal **pH** >4.5.
- Fishy **odor** when (KOH) is added to a sample of vaginal discharge.
- **Clue cells** on saline wet mount

Nugent's scoring system for diagnosis of bacterial vaginosis

Score	Lactobacillus morphotypes	Gardnerella and Bacteroides morphotypes	Curved gram-variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

0 to 3 is normal
4 to 6 is indeterminate
7 to 10 is indicative of BV

1 Schwebke JR et al. Obstet Gynecol. 1996
2 Bradshaw cs et al. J Clin Microbiol. 2005

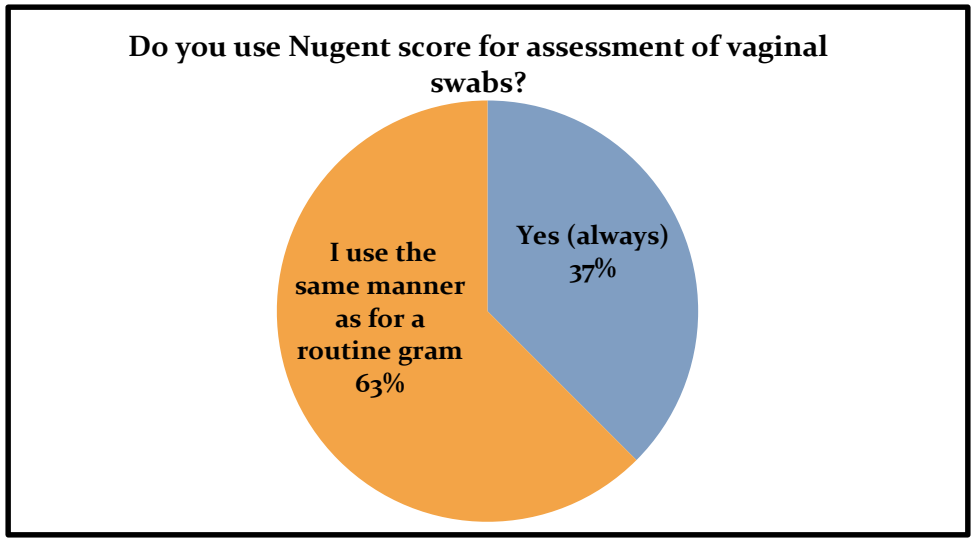
4.2.a Genital samples (vaginal samples for Bacterial vaginosis):

- Hay/Ison criteria **alternative to Nugent score in busy hospitals [2].**

	Lactobacilli morphotypes	Gardnerella morphotypes
Normal	Many	Few
Intermediate	Equal amount	Equal amount
BV	Few	Many

- **IDSA 2018**:
  - Gram stain (gold standard),
  - Vaginal culture has no role in BV Dx.

- **Survey result:**



1 Schwebke JR et al. Obstet Gynecol. 1996
2 Bradshaw cs et al. J Clin Microbiol. 2005

4.2.b Genital samples other than vaginal samples:

Urethra/cervix/genital ulcer:

- **IDSA 2018 :**
  - unnecessary for Dx cervicitis
  - has a role in Dx of chancroid, granuloma inguinale and (Gonorrhe a in males).

- **Gonococcal urethritis:**
  - Urethral swab should be **at least** 2 cm into the urethra and rotated 360 degrees!
  - American guidelines: ≥2 WBC
  - European guidelines: >5wbc
  - Intracellular diplococci.
  - Sensitivity: **38%** [1] (cut off 2 WBCs)
  - Specificity: **79%**

- Reports of ***N. meningitidis*** causing symptomatic urethritis and being initially mistaken for *N. gonorrhoeae* on Gram stain !!

- NAAT is still indicated to confirm the presence of *N. gonorrhoeae* and to **exclude coinfection** with *C. trachomatis*.

1 Orellana MA. Sex Transm Infect. 2012

Clinical impact of vaginal samples Gram stain:

- Gram stain is important for the diagnosis of asymptomatic BV.

- American College of Obstetricians and Gynecologists (**ACOG**) **2017** and **CDC**:

  - **Not routinely screen and treat all pregnant women** with asymptomatic BV→ possible benefit by preterm birth.
  - Screening and treatment are **recommended for females with gynecologic complications**
  - Reductions in postoperative infectious complications (**10% to 75%**) [1-2]

- Insufficient evidence to make a conclusion regarding screening for BV prior to IUCD.

1 Larsson PG, J Obstet Gynecol. 1992
2 Penney GC. Br J Obstet Gynaecol. 1998

4.3. CSF Gram stain:

- The sensitivity of gram stain in diagnosing CNS infection varies depending on the **organism and population**.

Pathogen	Sensitivity (%)[a]			
	Blood culture	CSF Gram stain	Latex agglutination test[b]	PCR
Haemophilus influenzae	25-90	25-65	78-100	72-92
Streptococcus pneumoniae	60-90	69-93	59-100	61-100
Neisseria meningitidis	40-60	30-89	22-93	88-94
Listeria monocytogenes	10-75	10-35	NA	NA
Streptococcus agalactiae	80-85	80-90	NA	NA
Streptococcus	60-65	66-73	NA	NA

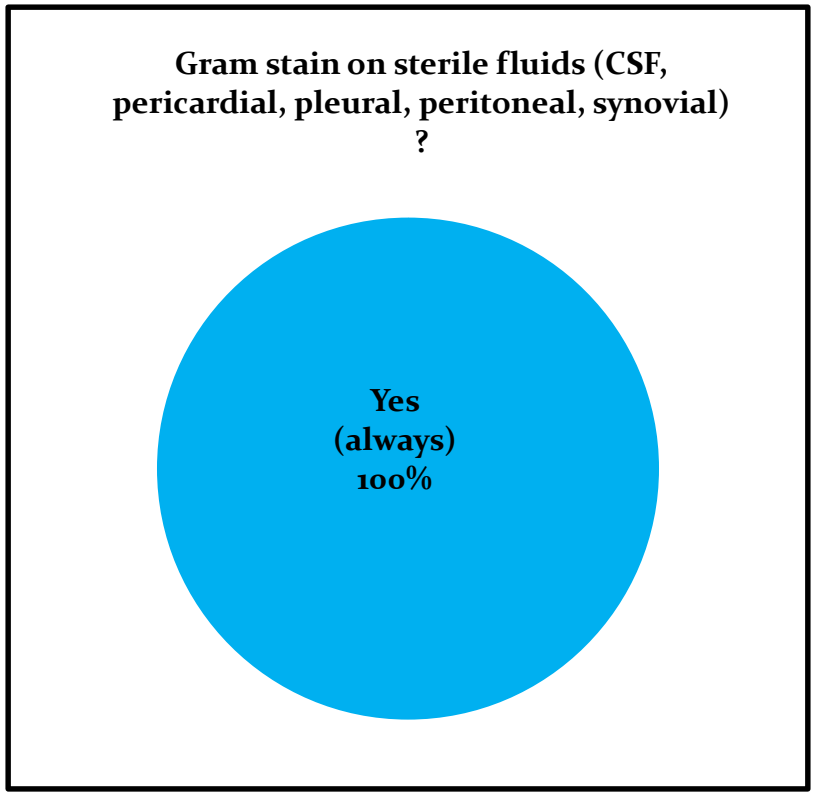
Brouwer MC et al. Clin Microbiol Rev. 2010

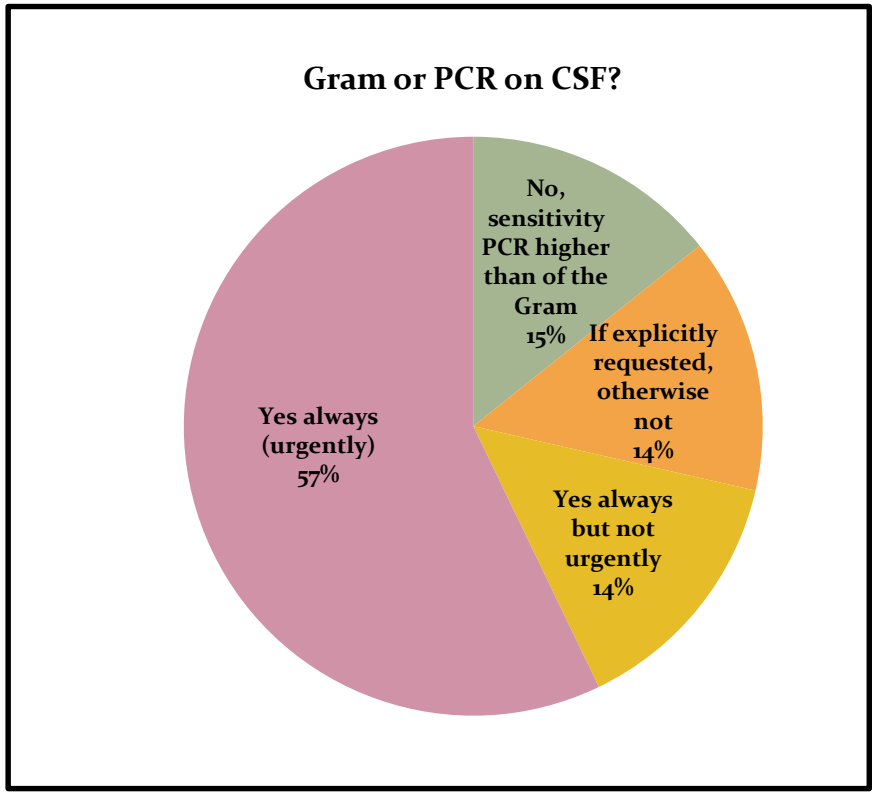
- **Possible alternatives for Gram stain in CNS infection**:
  - Multiplex FilmArray meningitis/encephalitis panel (BioFire) or in-house panels
  - Antigen detection (latex agglutination).

- **Latex agglutination test**:
  - IDSA 2018 : **not recommended for meningitis**
  - It may have some value in patient with negative gram stain and negative culture due to therapy after 48 hour incubation. It may be reserved for such cases only

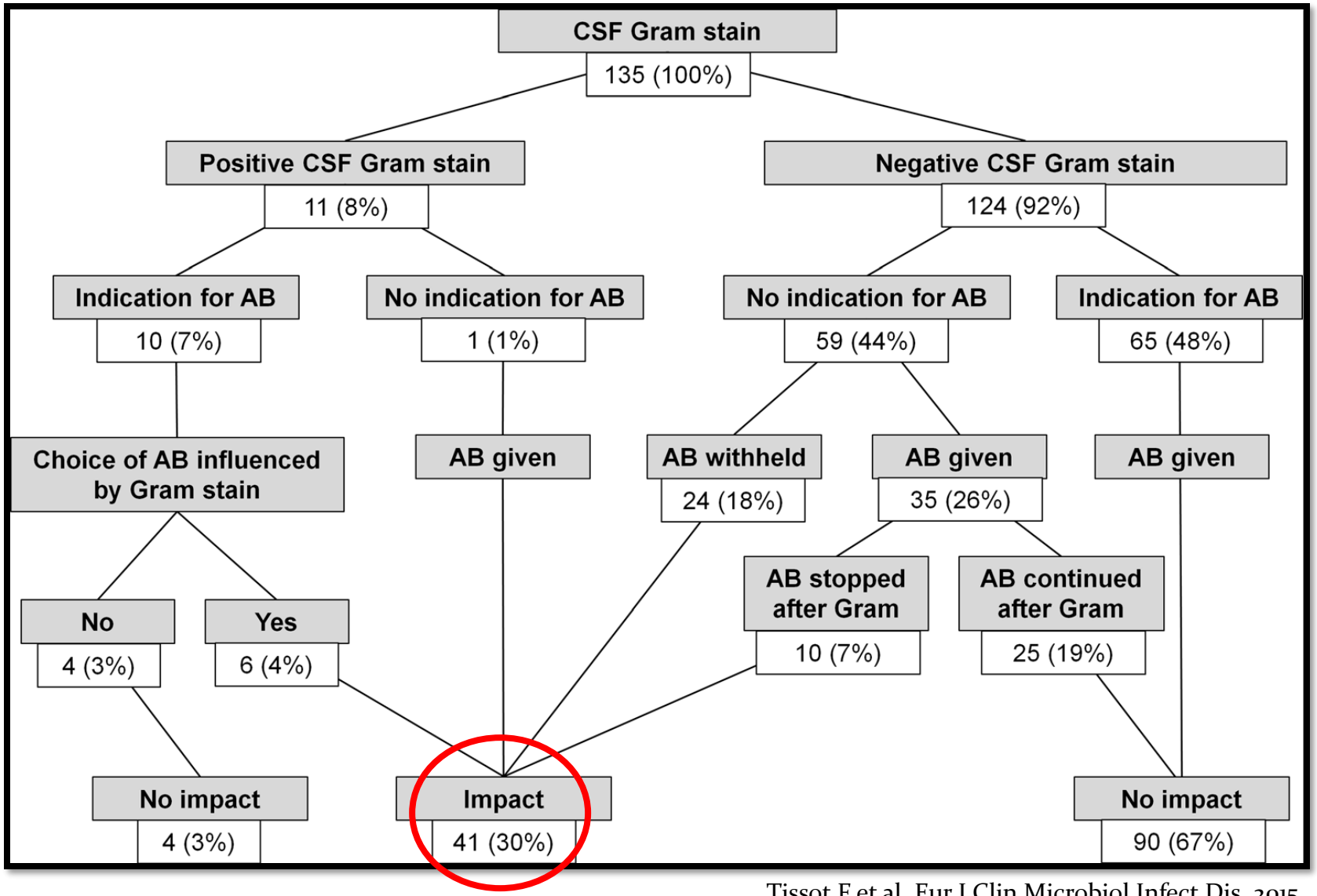
- **FilmArray** (IDSA 2018/UpToDate):
  - Highly sensitive and specific
  - It leads to increase pathogens that can be identified.
  - It does not depend on bacterial load
  - It is not affected by antibiotic exposure
  - It does not depend on experience of the examiner in Gram stain interpretation.
  - Superior to Gram stain in detection of co-infection of CSF
  - **not as alternative for culture**

Survey result:





Clinical impact of CSF Gram stain: 30% effect



Tissot F et al. Eur J Clin Microbiol Infect Dis. 2015

Clinical impact of CSF Gram stain:

- It seems that without multiplex PCR, the diagnosis of meningitis is a big challenge.
- This might support proposing **round-the-clock 24/24 CSF PCR** instead of gram stain or both.
- Both PCR and Gram stain may be needed when meningitis occur in a particular context such as trauma)→case reports
- Broad-spectrum antimicrobial therapy will be continued whether Gram stain result is positive or negative [1].
- PCR panel leads to more detected cases of meningitis, more targeted use of antibacterial and antiviral therapy especially in children [1].

1 Tissot F et al. Eur J Clin Microbiol Infect Dis. 2015

4.4. Diagnostic performance of synovial fluid Gram stain:

- Bram JT [1] et al.: (**septic arthritis-pediatrics**)

  (2018, 302 **pediatric** septic arthritis in an case control study):
  - Sensitivity: **40%** and much lower for gram neg.
  - Specificity: 97%

- Carpenter CR [2] et al: (**septic arthritis – adults**)
  - Sensitivity **30% to 70%**
  - Specificity up to 100%.

	Gram's stain sensitivity	Culture sensitivity
Non-gonococcal arthritis	50–70%	75–95%
Gonococcal arthritis	10–25%	10–50%
Tuberculous arthritis	20%	79%

Scott R. Brannan et al..J Emerg Med 2008

- Updegrove GF [3] et al. / Morgan PM [4] et al : (**prosthetic joint infections**)
  - Sensitivity: **7%-27%**
  - NPV: 57%-89%.

1 Bram JT et al. J Pediatr Orthop. 2018

2 Carpenter CR, JM. Acad Emerg;2011 Med 18

3 Updegrove GF et al. J Shoulder Elbow Surg. 2015.

4. Morgan PM et al. J Bone Joint Surg 2009

Clinical impact of synovial fluid Gram stain:

- **IDSA 2018**:
  - Synovial fluid should be submitted for Gram stain, and culture.
  - Gram stains are **not recommended** for the diagnosis of prosthetic joint infection.
- **Trampuz PJI**: not mentioned
- **IGGI/UpToDate**:
  - Empirical antibiotic therapy for septic arthritis is guided by results Gram stain.
  - Guidelines still recommend Gram stain for the diagnosis of septic arthritis or bursitis (high specificity).

- Gram stain is an **unreliable tool** for ruling out periprosthetic infection or septic arthritis because of the low sensitivity and low negative predictive value.

- The synovial **WBC and percentage of PMN** cells are required to assess the likelihood of septic arthritis before the Gram stain and culture test results are known .

4.4. Diagnostic performance of pleural fluid Gram stain:

- Poe RH [1] et al.:
  - Sensitivity: **18%**.
  - **Effusion after a bacterial pneumonia**.

- Barnes TW [2] et al.:
  - **2.5%**
  - This showed the low yield of Gram stained smears especially in the outpatient setting and in patients with free-flowing effusions (**not infectious**)

4.5. Diagnostic performance of pericardial fluid Gram stain:

- With the exception for some case reports, **no evidence** available over Gram stain utility and its diagnostic performance in pericarditis patients.

1 Poe RH et al. Chest. 1991
2 Barnes TW et al. Chest. 2005

4.6. Diagnostic performance of peritoneal fluid Gram stain in patient with spontaneous bacterial peritonitis :

- **Dx of SBP** depends on an increased peritoneal absolute **PMN greater than 250 cells/mm**
- Chinnock B [1] et al :
  - **Sensitivity: 10%**
  - Specificity: 97.5%
  - PPV: 48%
  - NPV: 81.3%
- Runyon BA [2] et al:
  - Sensitivity: **9% (all patients have SBP)**
- Case reports: SBP due to **Listeria monocytogenes**
- **Gram-positive rods on Gram's stain is mostly a contaminant such as *diphtheroids*, but it could be also *L. monocytogenes***

1 Chinnock B et al. Ann Emerg Med. 2009
2 Runyon BA et al. Gastroenterology. 1988

Clinical impact of body fluids Gram stain:

- **IDSA 2018/Leber 2016:**
  - Gram stain is recommended on all sterile body fluids

- The ultimate diagnosis of empyema (pleural fluid), SBP (ascitic fluid), and bacterial pericarditis (pericardial fluid) **depends mostly on the analysis of the fluid**.

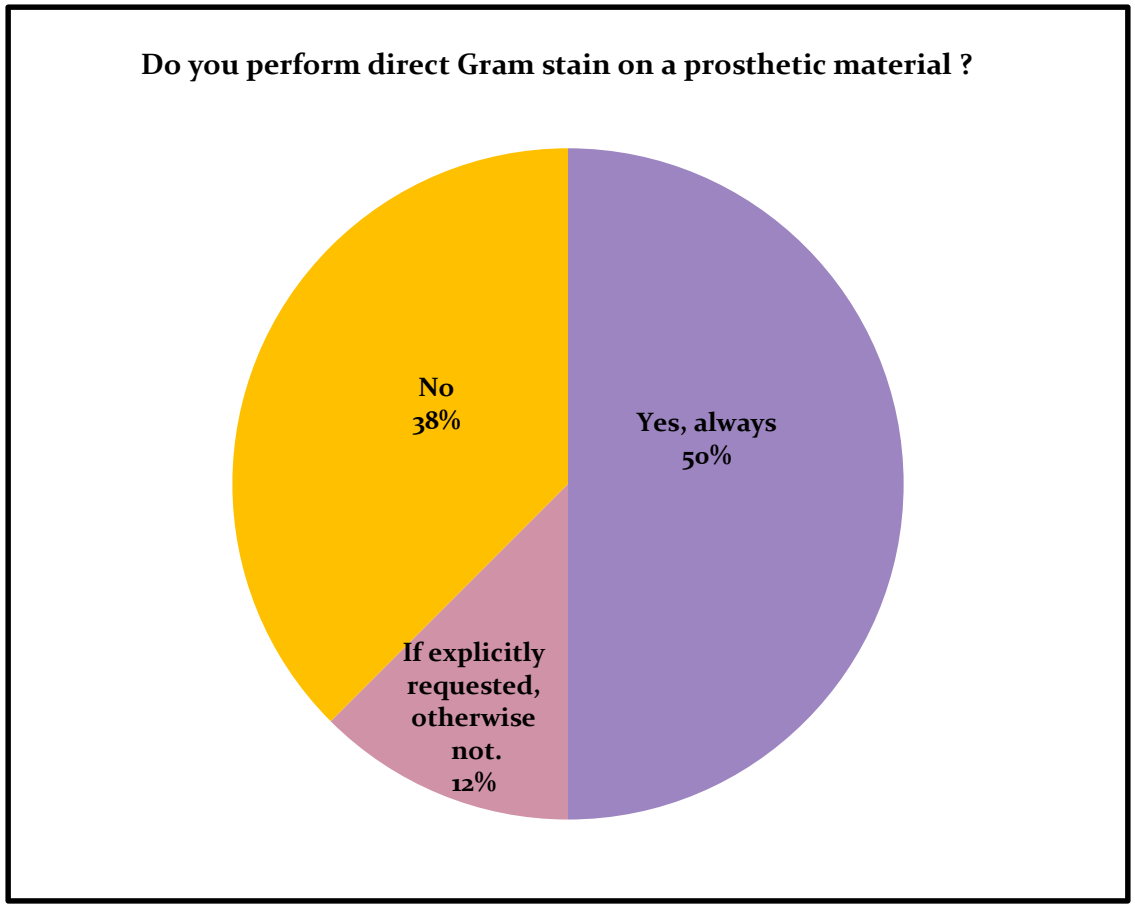
- In practice, Gram stain result follows nearly always the cell count result.

- **Gram stain of body fluids can give misleading information.**

4.7. Diagnostic performance of divers material Gram stain:

4.7.a.Prosthetic joint material:

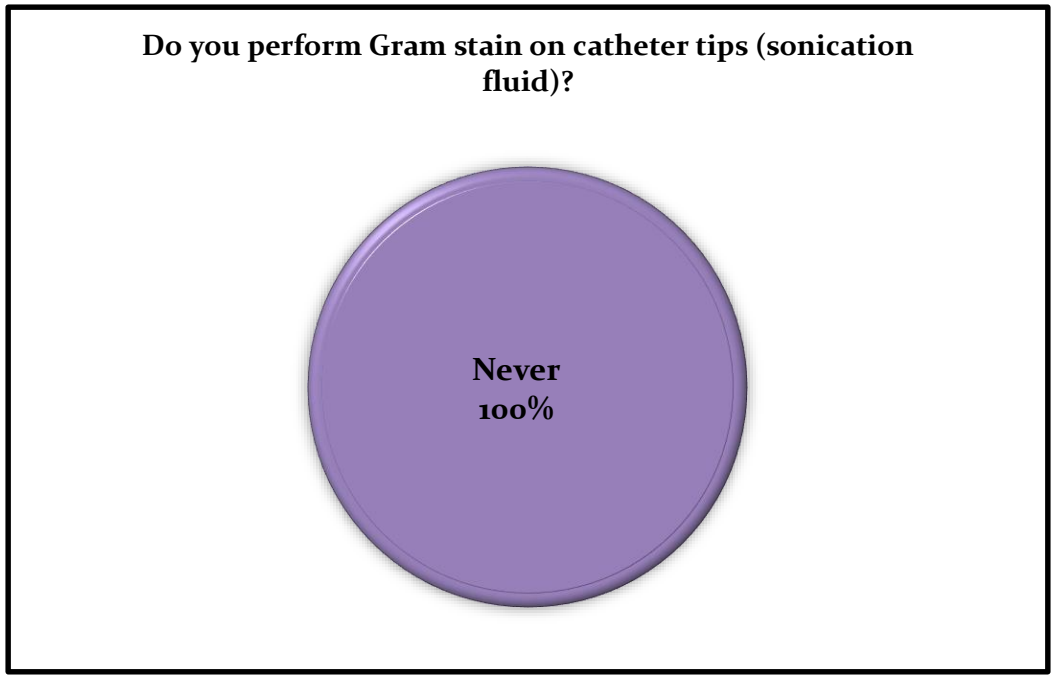
- **No data available** about the diagnostic performance of prosthesis Gram stain in patients with prosthetic joint infection.



4.7. Diagnostic performance of divers material Gram stain:
4.7.b. Catheter tip:

- **Leber 2016/IDSA 2018**:
  - **Not part of the standard protocol for catheter tip samples.**

- **Guemba M.** et al. [1] :
  - 20 Oil-immersion fields should be screened
  - It is **impossible to be implemented in a busy laboratory**



1 Guembe M. Eur J Clin Microbiol Infect Dis. 2015

4.8. Diagnostic performance of wounds Gram stain

- Kaftandzieva A. [1] et al:
  - Sensitivity: **38%**
  - Specificity: 90%
  - PPV: 83%
  - NPV: 54%

- Elsayed S. [2] et al (burn wounds):
  - kappa: **0.32**

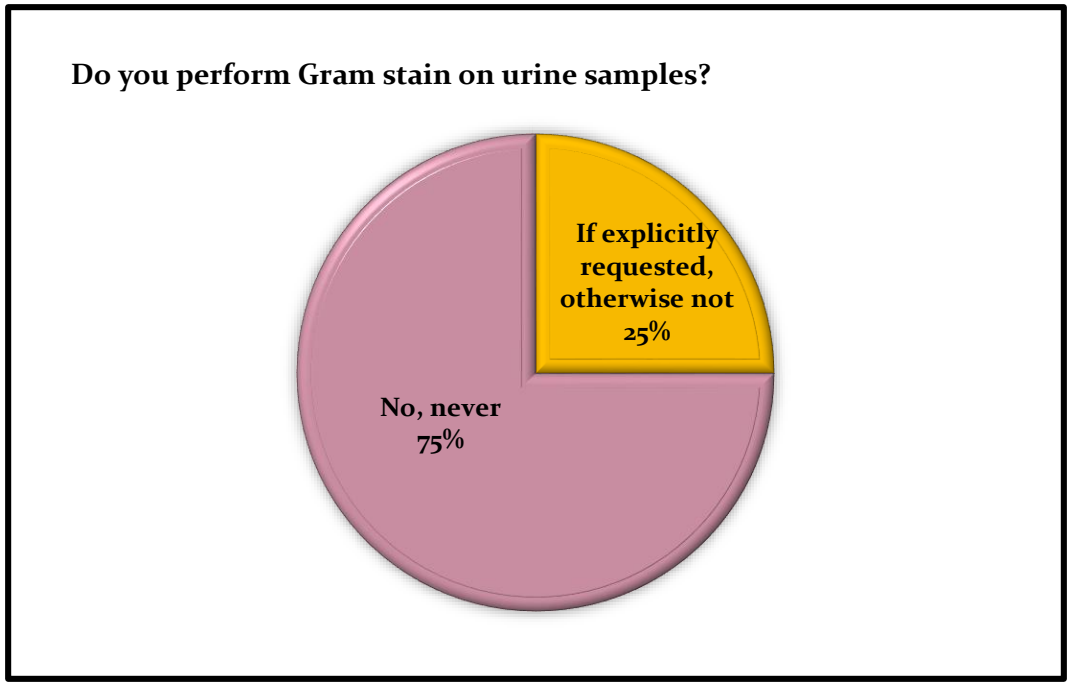
Clinical impact:

- All wounds are **colonized with microbes.**
- **Gram stain information is not sufficient** to guide the choice of AB therapy.
- Empiric therapy should be a broad-spectrum antibiotic with coverage of gram-positive cocci as well as the expected flora at the site of operation.
- Culture of wound specimens should guide the AB therapy.

1 Kaftandzieva A et al. Macedon of Med. Science. 2012
2 Elsayed S. et al Arch Pathol Lab Med. 2003

4.9. Diagnostic performance of urine Gram stain:

- **IDSA 2018**:
  - It is **not the appropriate method to detect PMNs in urine.**
  - It can be ordered as an option for detection of high numbers of GNR in suspected urosepsis.
  - Infections with lower bacterial concentrations than **10^5 CFU/mL** may not be detected.
- **Murray PR** [1] et al.:
- It is **too insensitive** to be used to identify infected patients



1 Murray PR et al. , J Cli Microbiol, 1987

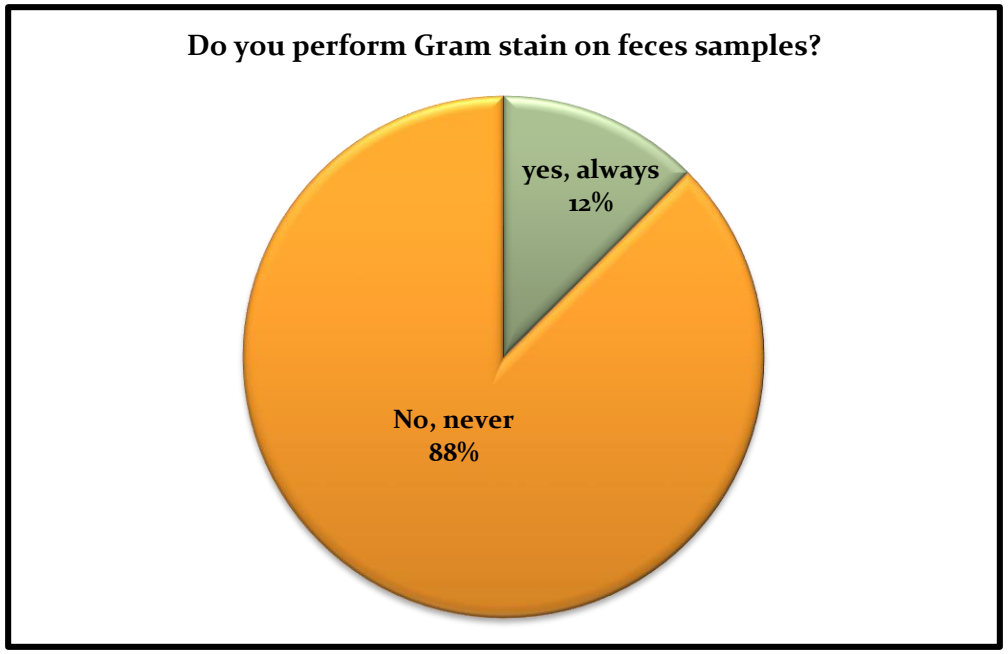
Clinical impact of urine Gram stain:

- It is labor intensive and requires experience.
- A prospective American study (312 child with UTI):
  - Empirical therapy was prescribed before the urine Gram stain result was known in 40 (49%) patients and after in 42 (51%) patients.
  - The **antibiotics chosen did not differ between the two groups** (P=0.81)

4.10. Diagnostic performance of feces Gram stain:

- **IDSA 2018**:
  - It **has not been mentioned** by laboratory diagnosis for GIT infection.

- **Leber 2016**:
  - It has a **very limited clinical value** (*Campylobacter*).



4.10. Diagnostic performance of feces Gram stain:

- The intention from direct Gram smear for stool samples is **to detect WBC** and bacteria.
- No study has compared the relative number of leukocytes found with each type of infection.
- Sensitivity (WBC in stool): **50% to 60%** for gastroenteritis [1] [2]
- **Sensitivity: 14%** for *Clostridium difficile* colitis [3]

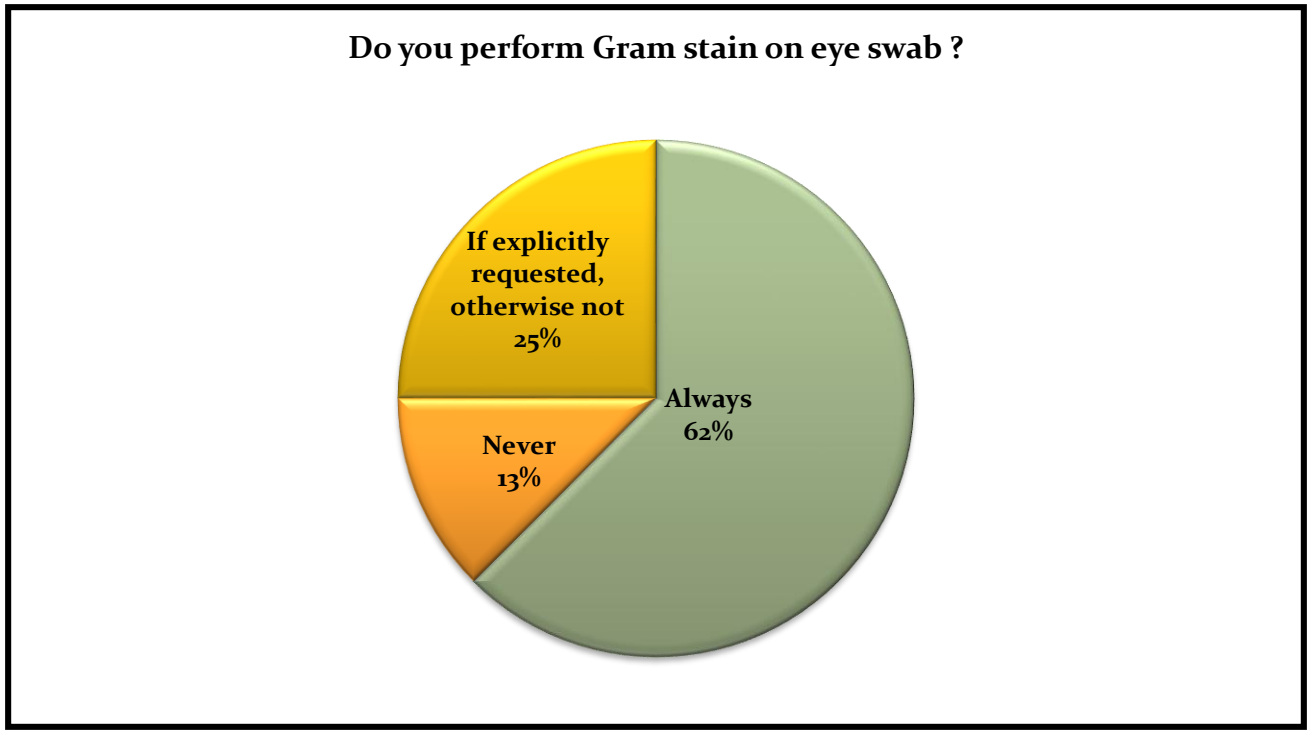
Clinical impact of feces Gram stain:

- Empiric antibiotic therapy **(azithromycin)** will be indicated when patients has a severe disease, or at high risk for Cx.
- WBC in stool samples is not linked to type infection (*Salmonella, Shigella, Campylobacter and Yersinia)*
- Clinical suspicion of *C. difficile*: results of antigen/toxin tests are known on the same day.

1 Ruiz-Pelaez JG et al.Pediatr Infect Dis J. 1999
2 Savola KL et al. J Clin Microbiol. 2001
3 Shanholtzer CJ et al.. *J Clin Microbiol*. 1983

4.11. Diagnostic performance of eye swab Gram stain:

- **IDSA 2018/Leber 2016**:
  - It is useful in the Dx of conjunctivitis.
  - It is also useful in the Dx of keratitis and endophthalmitis (inner eye specimens).



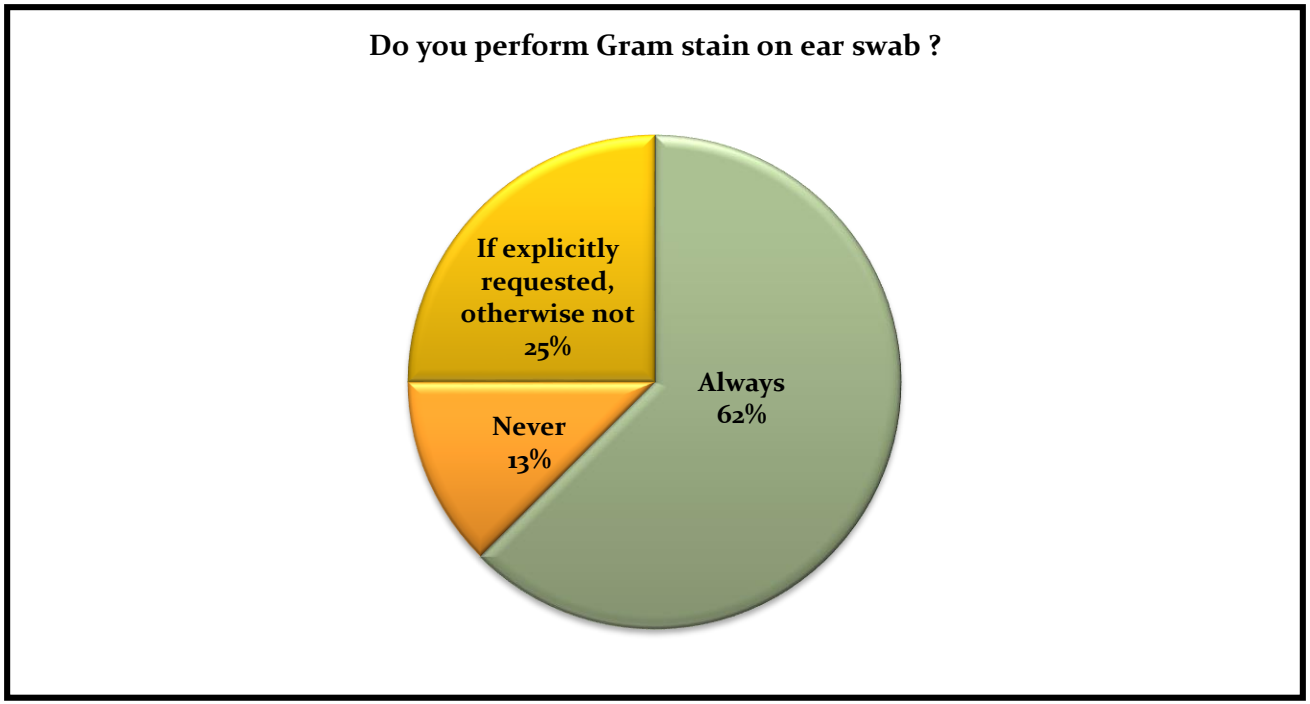
Neisseria conjunctivitis !!
PMN & GND

→ **Systemic + topical AB**

→ **Prophylaxis for close contacts**

4.12. Diagnostic performance of Gram stain from upper respiratory tract :

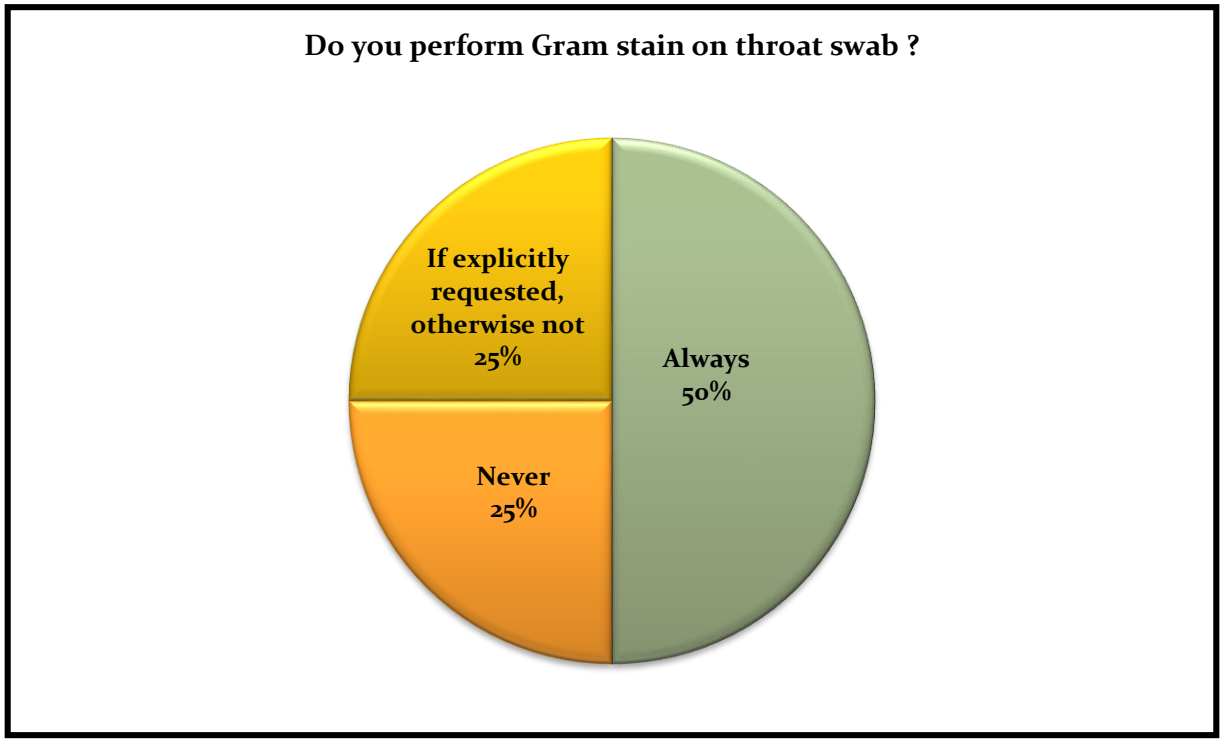
- **IDSA:** Gram stain is recommended by :
  - Otitis externa / otitis media
  - Mastoiditis
  - Sinusitis



- WBC higher in patients with culture-positive AOM than in those with culture-negative AOM and in those *S. pneumoniae* AOM.
- **No evidence** for clinical impact.

4.13. Diagnostic performance of mouth swab Gram stain:

- **IDSA 2018:**
  - Vincent angina
  - Peritonsillar cellulitis or abscess



- Vincent angina: **clinical Dx, Gram stain may support the diagnosis, culture not recommended**
- Peritonsillar cellulitis or abscess: **no evidence** for utility or impact.

5. Diagnostic performance of indirect Gram stain:

5.1. Biopsies:

Heart valves from patients with infective endocarditis:

- Morris AG [1] et al,2003 .:
  - Valves were **seldom culture positive** after 50% of the standard AB therapy
  - Gram stain were **positive for >60%** of patients still receiving AB.
  - The microbiology Gram stain was more likely to be positive than histopathology Gram stain (74% vs. 63%; *P* <.0001)

- **Jung J** [2] et al. :
  - 24% had organisms seen on vegetation Gram stain but not cultured.

Clinical impact :

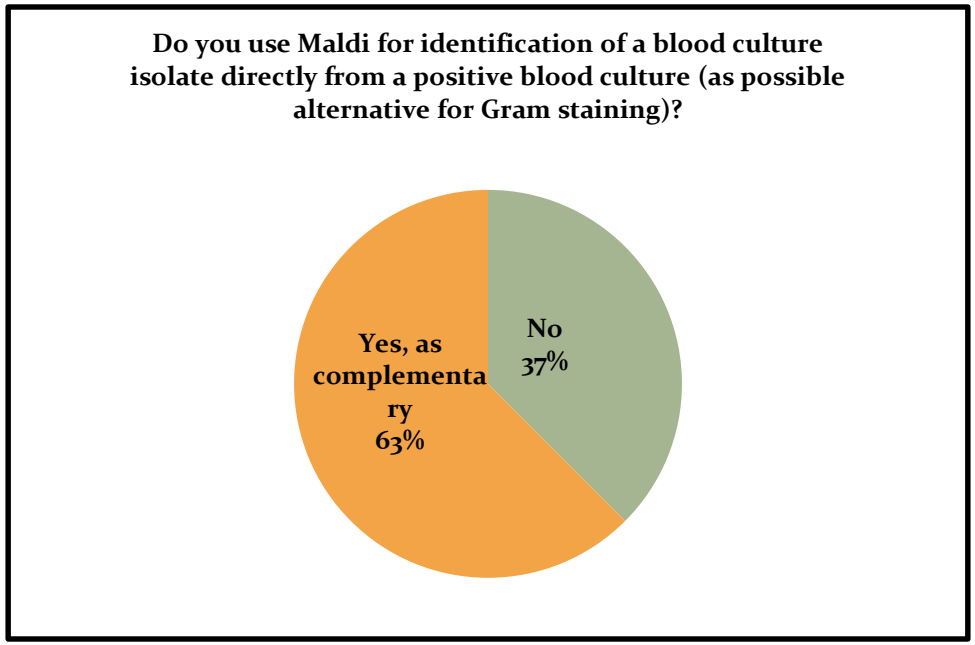
- A positive microbiology Gram stain has been dropped from modified Duke criteria.
- Modified Duke criteria include the positive **HISTOLOGICAL Gram stain**
- Positive microbiological Gram stain does not mean active infection.
- There is considerable time delay between vegetation sterilization and disappearance of organisms (**culture should be the index).**

1 Morris J et al. . Clin Infect Dis. 2003
2 Jung J et al. Thorac Cardiovasc Surg. 1975

5.2. Diagnostic performance of blood culture Gram stain:

- Hautala T [1] et al.
  - the knowledge of gram stain results and where the infection was occurred **allow accurate empirical AB therapy**

- The **Q-probes** study [2] :
  - gram stain reporting for blood stream infection was usually correct.
- Possible altenative:
  - Maldi-Tof
  - Molecular assays
  - Immune chromatographic lateral flow assay



Clinical impact:

- **No alternative to Gram stain** in patient with sepsis.
- Gehring T [3] et al:
  - The **clinical benefit** of immediate reporting (24/24)of Gram stain results, **especially in patients with fungus in the blood culture.**

1 Hautala T et al. Int J Antimicrob Agents. 2005
2 Schifman RB et al. Arch Pathol Lab Med. 2015
3 Gehring T et al. Eur J Clin Microbiol Infect Dis. 2019

6. Organizational impact:

- Shortening the duration of hospitalization?
- Reduced time spent by medical / paramedical care providers?
- Reduced use of other staff and / or non-staff resources?

No evidence

7. Financial impact:

- **Financial cost:**
  - Reagents & material (
  - Cost machin
  - Personnel
- **RIZIV reimbursem**
  - 126184 B70: with o
  - 126836 B90: with double staining
  - 549555 B400: CSF with double staining

Also crystals, eosinophiles in fluids and other parameters from sperma examination

- RIZIV reimbursement for B70, B90, B400 in 2018→ ✖

- **Total number direct Gram stained smears in 2018 in lmelda:**
  - **7.351 direct**
- 369 (CSF Gram stain):
  - 369 x 400 x 0,031254 = **4, 613**
- 6,982 (other than CSF):
  - 6,982 x 70 x 0, 031254= **15, 275**
  - 6,982 x 90 x 0, 031254= **19,639**

19,904 euro

Overview:

Sample	Gram	No Gram	?
Respiratory			✓ Guidelines?
Genital	✓ (for BV)		
Wounds		✓	
Eye swab	✓ (If no PCR for *Neisseria*)		
Ear swab		✓	
Mouth swab		✓	
Nose swab		✓	
Urine		✓	
Feces		✓	
Biopsies		✓	
Synovial fluid	✓ (septic arthritis)	✓ (PJI)	
Pleural/ pericardial fluid			✓
Peritoneal fluid			✓ (Listeria SBP?)
Cerebrospinal fluid	✓ (If no PCR/meningitis in a special context)		
Blood culture	✓		

❖ Conclusion:

- The clinical utility of Gram stain for most of microbiological specimens does not warrant the time or cost it requires. Gram stain can be considered as a valuable test:
  - **Direct :**
    - Vaginal samples to detect asymptomatic **bacterial vaginosis**, which is important for female who will undergo a gynecological procedure.
    - According to **IGGI/UpToDate**: the initial choice of empiric antimicrobial therapy for **septic arth**. is guided by the result of Gram stain→ **in practice ?!!**
    - **CSF** by suspected **meningitis**, if PCR M/E panel is not available or in special context.
    - ***Neisseria* conjunctivitis** or ***Neisseria* urethritis** in the absence of NAAT.
  - **Indirect :**
    - Positive blood culture, in order to guide the choice of empirical therapy.

❖ To do Imelda:

- Discuss with the clinicians the possibility of cancelling Gram stain when not needed:
  - Non sterile samples :
    -Wounds
    -Genital other than vaginal samples for BV
    -Upper and lower respiratory tract samples
  - Synovial fluid/biopsy Gram stain in periprosthetic joint infection

- Participation in INSTAND EQC
- Inter-individual testing more frequent.
- Reporting Gram for BV in a score system in LIS.

Questions?