New diagnostic methods

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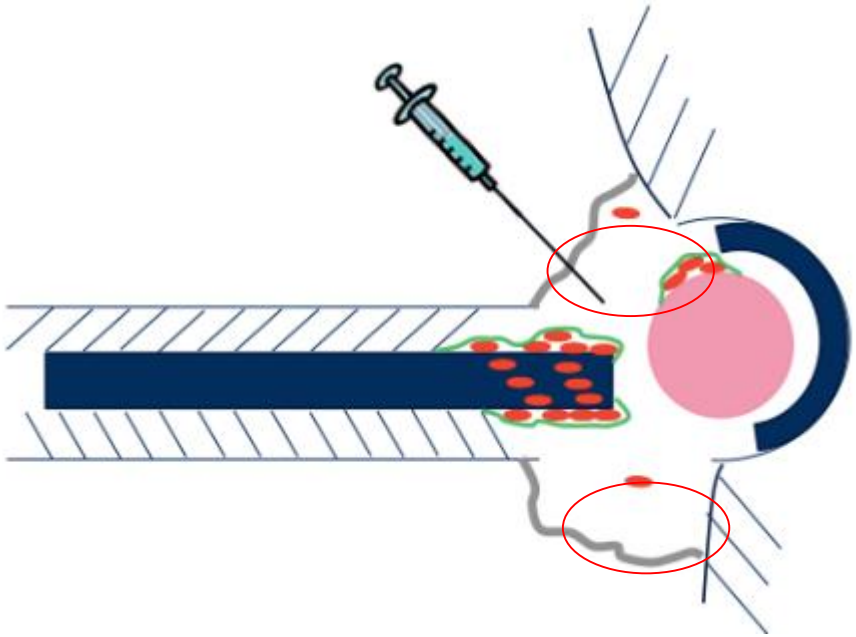
Preoperative diagnostics

Joint puncture

Cell count of synovial fluid

Analysis of synovial fluid of 142 patients with prosthetic failure

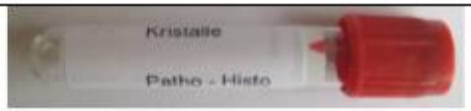
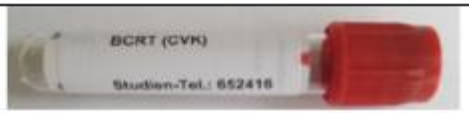
Diagnostic test	Aseptic failure (n = 65)	PJI (n = 77)	sensitivity (%)	specificity (%)
Cell count (n = 103)	**2/47**	**48/56**	**82%**	**96%**
Culture (n = 142)	1/65	40/77	52%	98%



Morgenstern C. et al, DMID, 2017

Arthrocentesis kit

Arthrocentesis-kit

Priorität	Röhrchen	(mindest-) Volumen	Sonstiges	Zweck	Ziel	
1	EDTA (Lila)	1 ml	Zellzahlanmeldung per S… sorgfältig schütteln!!	Zellzahl	**Verteilerlabor** Rohrpost: 1213	
2	BK-Flasche	mind. 1ml	MiBi-Schein	MiBi	**MiBi**	
3	Rot	0,5 ml	Patho-/Histo-Schein	Kristalle	**Patho-/Histologie Verteilerlabor** Rohrpost: 1213	
4	NATIV (in der Spritze)	1 ml	MiBi-Schein „NATIV“	MiBi	**MiBi Verteilerlabor** Rohrpost: 1213	
5	Rot	1,5 - 2 ml	Bitte unterschriebenes Einwilligungsformular mitschicken	Kalorimetrie + PCR	**Ortho-Op Dispatcher** Rohrpost: 1605 **mit unterschriebenem Einwilligungsformular!!**	

Cell count

Microbiology (cultures)

If **aspirated synovial fluid volume <5 ml** distribute the obtained synovial fluid **according to the priority column** (otherwise vials can be completely filled up)

Which cutoff is most accurate?

Author	Patients (No. & joint)	Variable[1]	Sensitivity	Specificity
Schinsky MF, 2008	55/201 (hips)	Lc count **>4200**	84	93
		PMN **>80**	84	82
Cipriano CA. 2008	146/810 (hips &	Lc count **>3450**	91	93
Zmisto				
Trampu				
Dinnee	&knees)	PMN **>65%**	90	87
Ghanem E, 2008	161/429 (knees)	Lc count **>1100**	91	88
		PMN **>64%**	95	95

Cut-off for prosthetic joint infections:

>2000 leukocytes/µl

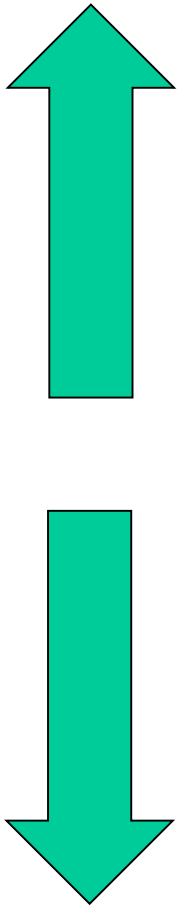
or

>70% granulocytes

[1] Lc count = leukocyte count (/µl)
PMN = polymorphonuclear cells (%)

Trials which applied sonication

Leukocyte count: not always reliable



Potentially false high

- 6 weeks postoperative
- rheumatologic disease
- after trauma/periprosthetic fracture
- relapsing dislocations
- metallosis

Potentially false low

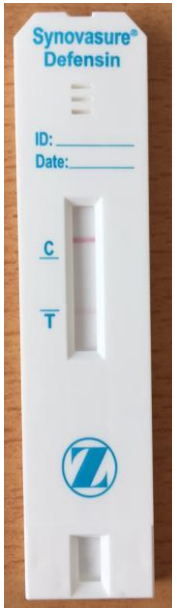
- sinus tract
- Low virulent pathogens?

Alternative tests in synovial fluid?

Test		Sensitivity	Specificity
Alpha-defensin		**67%**	**93%** Kasparek MF, JA 2016
D-Lactate		**94%**	**98%** Karbysheva S

Alpha defensin

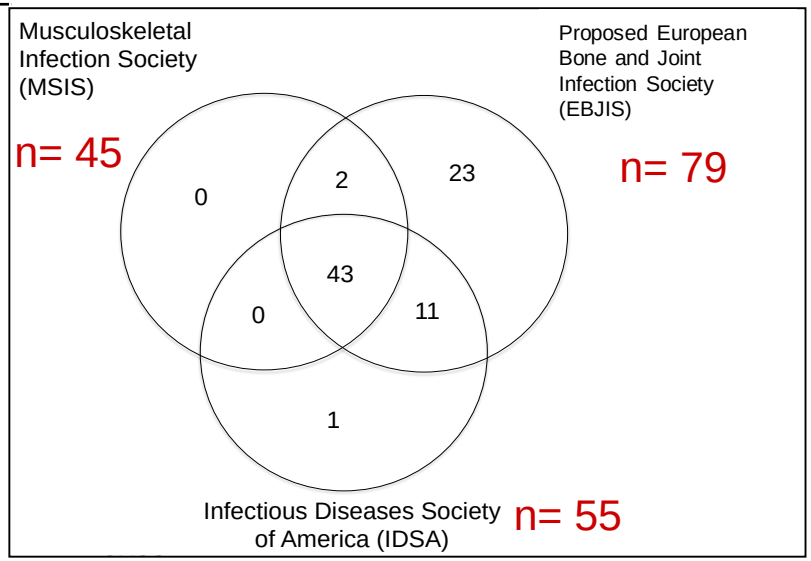
- **Alpha defensin** is an antimicrobial peptide released by neutrophils
- Previous studies showed **high accuracy** of quantitative determination of alpha defensin (ELISA) for discrimination between aseptic failure (AF) and periprosthetic joint infection (PJI)
- Qualitative bed side lateral flow test is based on alpha defensin concentration in synovial fluid for detection of PJI



	Sensitivity	Specificity	References
Quantiative alpha defensin (ELISA)	97-100%	95-100%	Bingham J, CORR 2014 Deirmengian C, CORR 2014 and 2015 Frangiamore SJ, J Arthroplasty 2016 Wyatt MC, JBJS 2016 Bonanzinga T, CORR 2017
Qualitative alpha defensin	67-77%	82-94%	Kasparek MF, J Arthroplasty 2016 Sigmund IK, BJJ, 2017 Suda AJ, Int Orthop, 2017

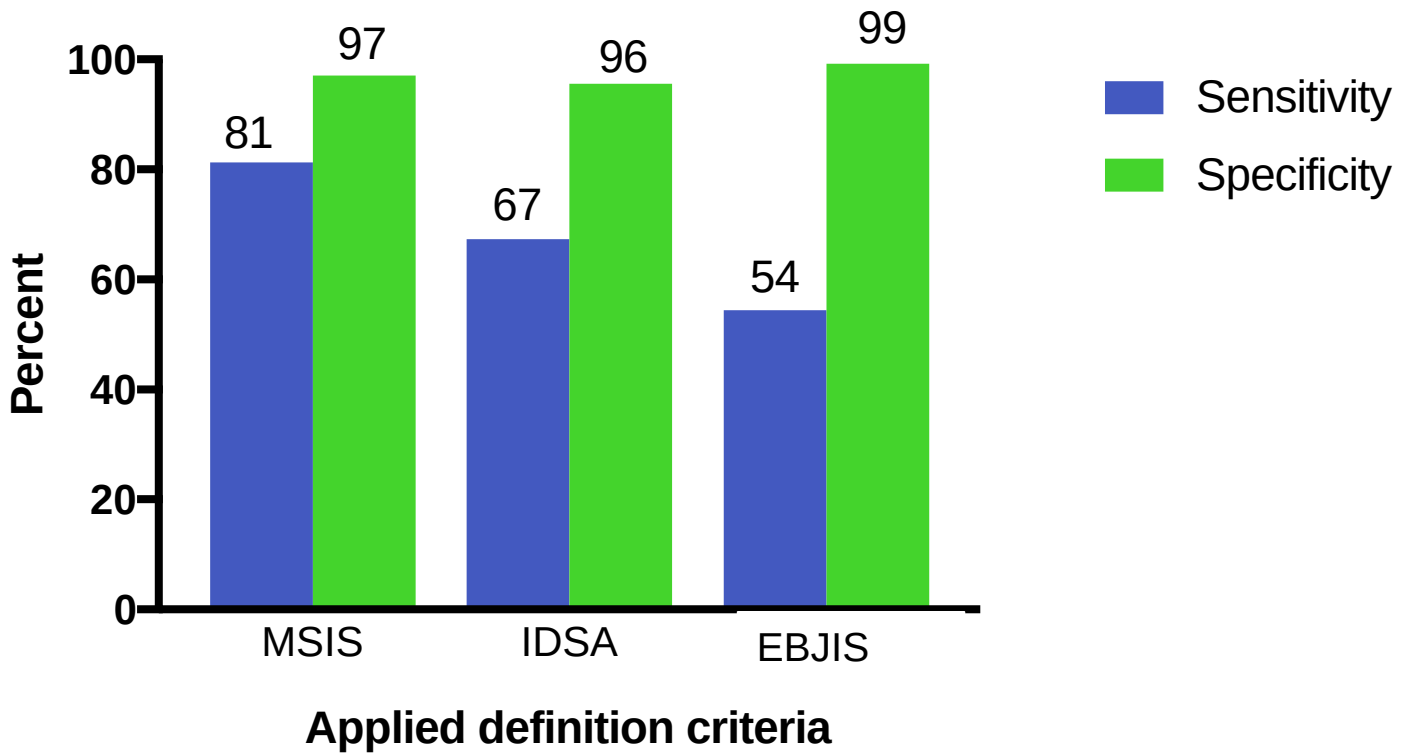
Demographics and infection characteristics

Characteristic	All patients (n=212)
Median patient age (range)	70 years (41-94)
Female gender, no (%)	106 (50)
Joint	
Knee	151 (71)
Hip	61 (29)
Timing of joint aspiration after primary surgery, no. (%)	
Early (<3 months)	33 (16)
Delayed (3-24 months)	79 (37)
Late (>24 months)	100 (47)
Patients undergoing revision surgery after joint aspiration	146 (69)



Renz N., JBJS 2018

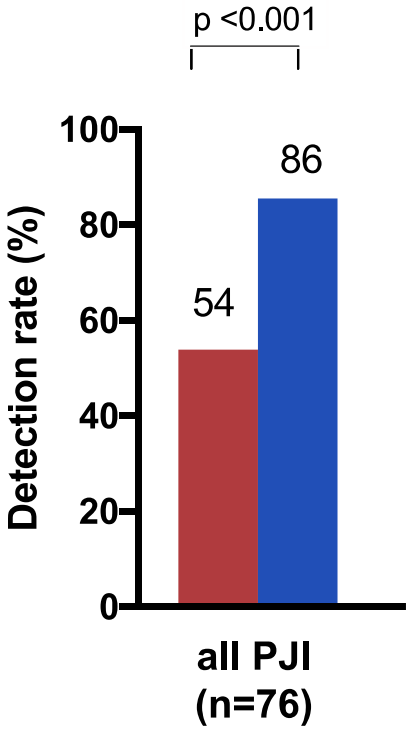
Performance of ADLF test



Applying proposed EBJIS criteria, which allow detection of low-grade infections, sensitivity of alpha defensin lateral flow test was 54% with a high specificity of 99% → no screening, but a confirmatory test

Renz N., JBJS 2018

Comparison with leukocyte count



PJI cases (based on EBJIS)

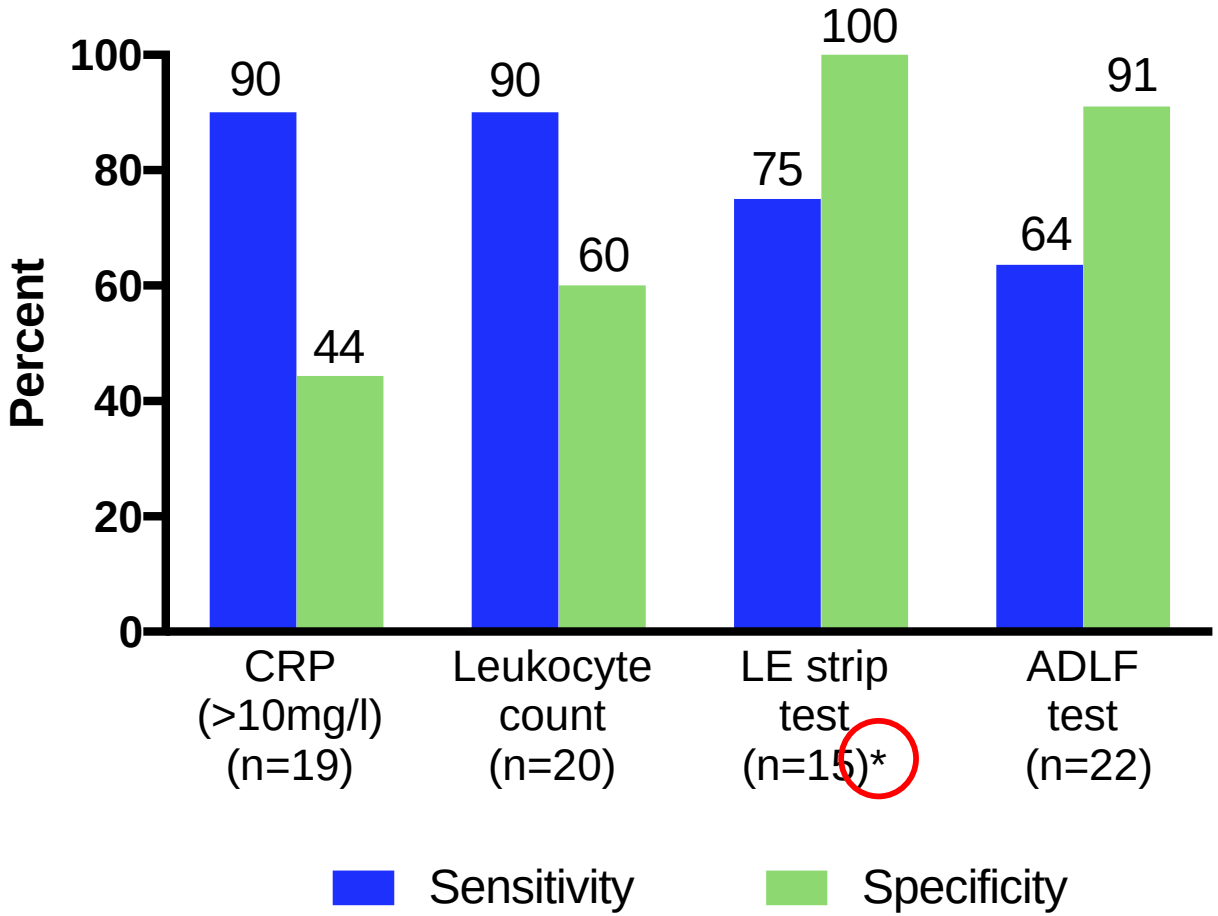
Alpha defensin lateral flow test
(result within 10 minutes)

Leukocyte count
(result within 2-4 hours)

Applying proposed EBJIS criteria, leukocyte count showed significantly higher sensitivity than alpha defensin, especially in chronic infections

Renz N., JBJS 2018

Performance in early postoperative infections



*12 tests were not readable due to blood contamination

Renz N., accepted JBJS 2018

Conclusion

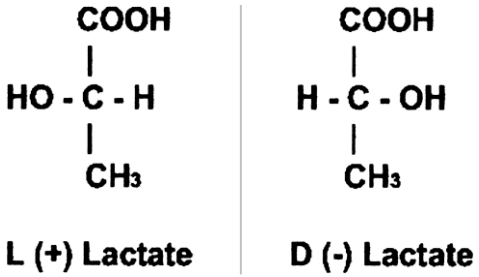
Alpha defensin lateral flow test is **not reliable** as **screening test**, however, it may be **useful** as **confirmatory test,** especially in early postoperative situations

New test

Lactic acid

L-lactate is constantly produced during metabolism and exercise

D-lactate is produced by bacteria as a product of bacterial fermentation



L. Szalay 2003; Sh.M. Smith 1994

Wellmer A. *2001; Gratacós J. 1995*

Method

Prospective multicentric study

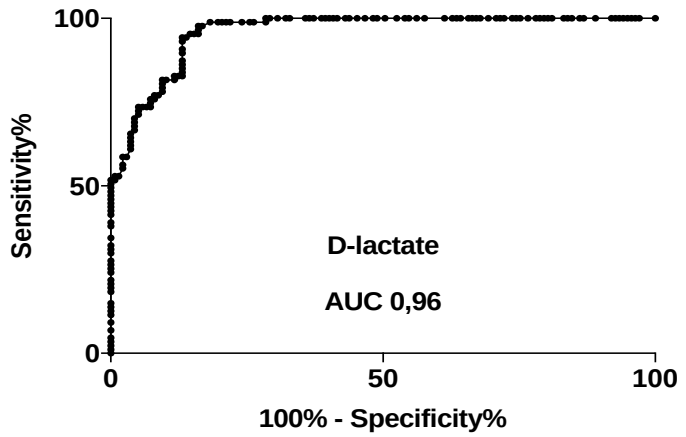
Manufacturer A
(SIVILAL, Belarus)

Manufacturer B
(SIGMA, USA)

	manufacture A		manufacture B	
	AF (n=137)	PJI (n=87)	AF (n=35)	PJI (n=29)
Age, median [range],yrs	65.4 (33-94)	59 (25-87)	68 (46-93)	72.7 (46-89)
Male gender, no. (%)	51 (37)	49 (63)	19 (54)	16 (55)
Affected joint, no. (%) - knee - hip	 83 (61) 54 (39)	 41 (56) 47 (44)	 21 (60) 14 (40)	 18 (62) 11 (38)

Results

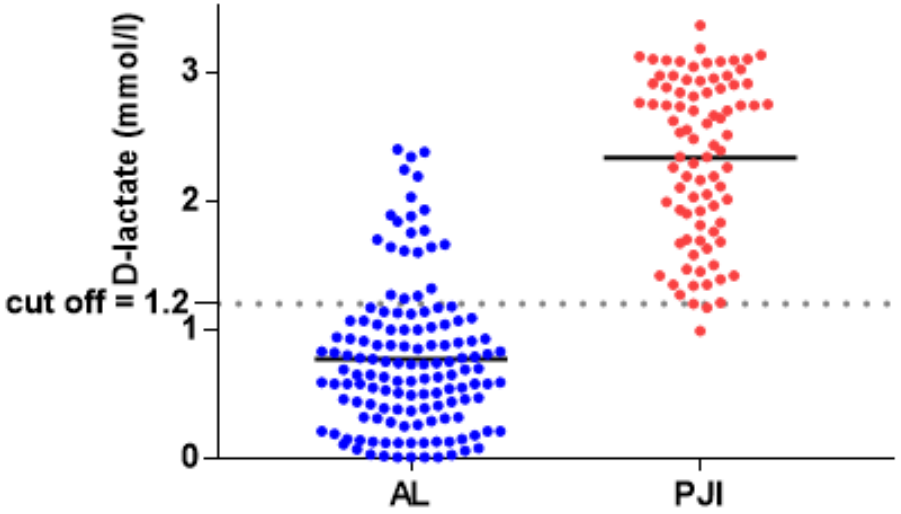
we determined optimal cutoffs and diagnostic accuracy



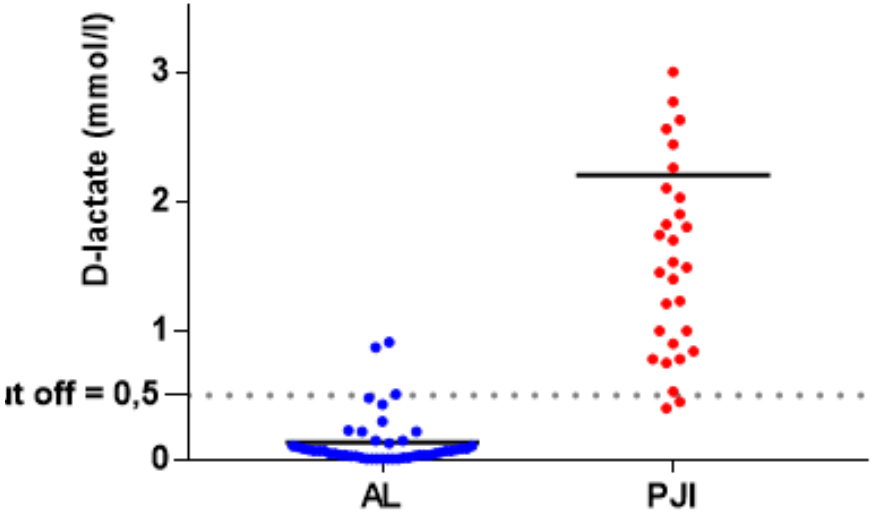
	Cutoffs	Sensitivity	Specificity	+PV	-PV
D-lactate (A), mmol/l	**1.2**	**98%**	**84%**	**79%**	**98%**
D-lactate (B), mmol/l	**0.5**	**94%**	**90%**	**92%**	**92%**

D-lactate Results

Manufacturer A



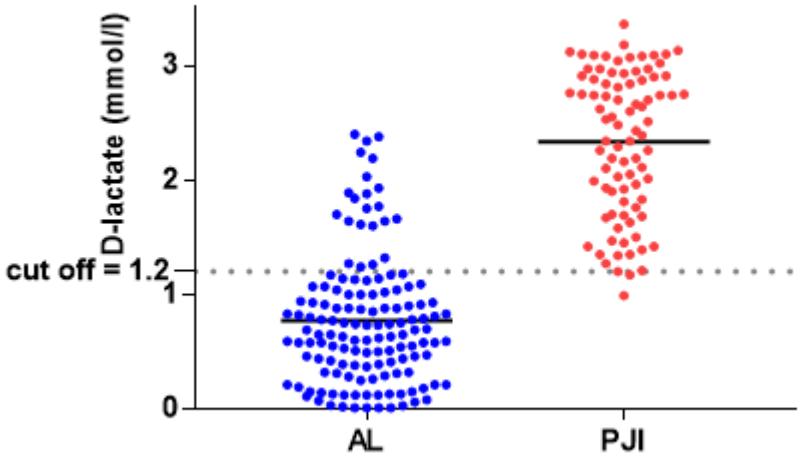
Manufacturer B



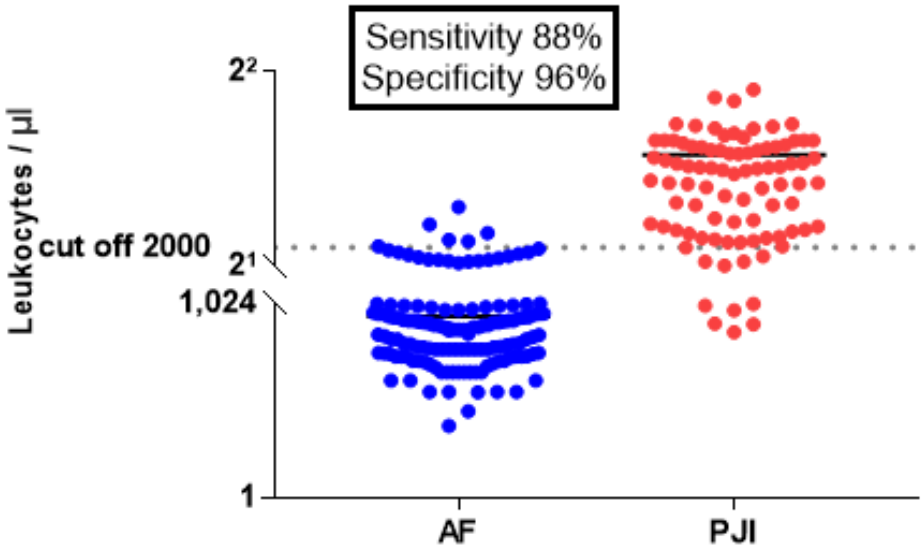
✓Irrespective of the kits, concentration of SF D-lactate was significantly higher in patients with PJI compared to those with aseptic failure (p<0,0001)

Results

D-lactate



Leukocytes



✓SF D-lactate test had better sensitivity to confirm PJI (98% and 94%) compared to leucocytes (88%)

Weakness of the test

Low specifisity – 84% - methodological reason

✓ nonstereoselective colorimetric enzymatic assay with low specificity

Outlook

✓ more stereospecific high performance methods
- **liquid chromatography** (HPLC) or
- **capillary electrophoretic** method

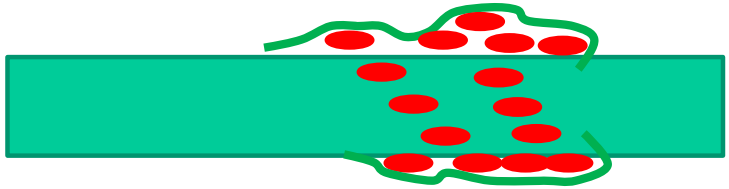
Conclusion

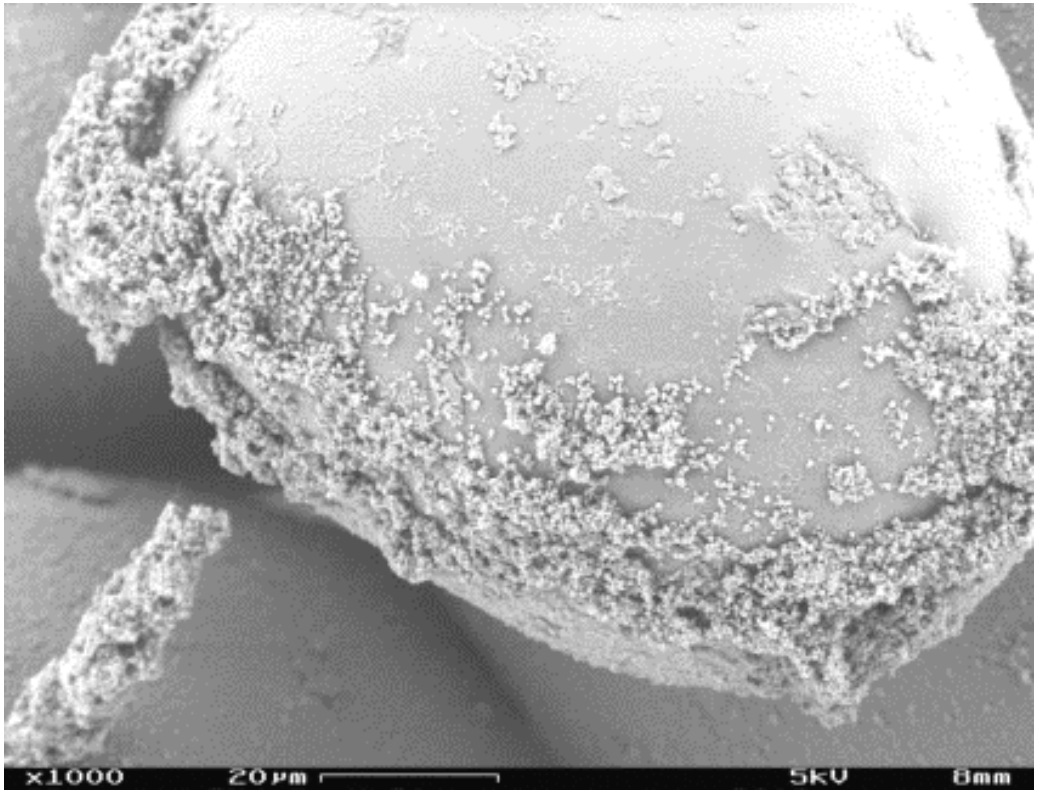
Cell count in synovial fluid is the most sensitive diagnostic tool in the preoperative setting and makes the diagnostic **joint aspiration** the most important diagnostic step

Intraoperative diagnostics

Explanted implants

Sonication – biofilm bacteria



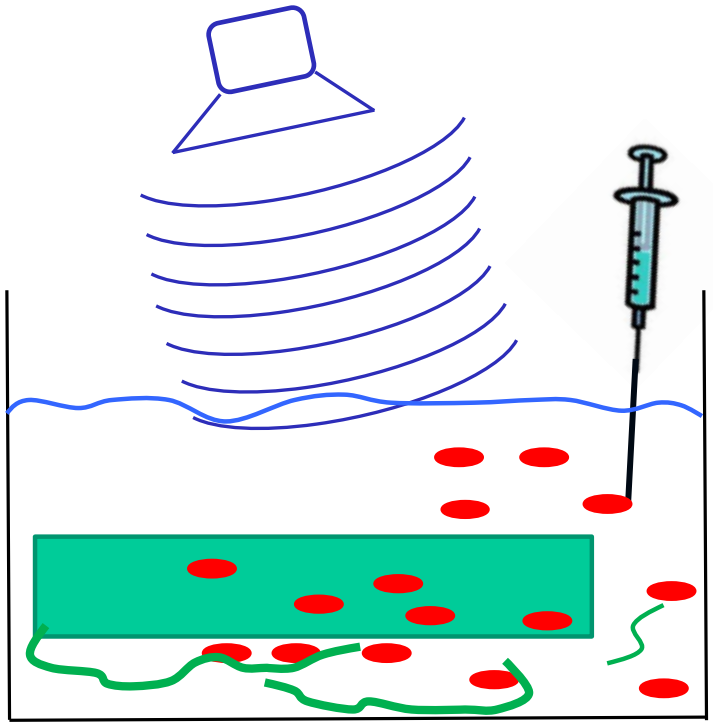


Sonication of implants

Removed implants

1 min, 40 kHz

0.5-1 W



Trampuz A et al. N Engl J Med 2007

Chemical dislodgement of biofilm

DTT-based method (MicroDTTect®)

Dithiothreitol (DTT)

- used in microbiology laboratories to liquefy the s
and thus may enhance detachment of bacteria from

76 patients
- 34 with aseptic loosening
- 42 with diagnosed PJI

	Sensitivity %	Specificity %
Sonication	71.4	94.1
DTT	85.7	94.1

Published online 2 July 2013 in Wiley Online Library
Use of Dithiothreitol to Improve the Diagnosis of Prosthetic Joint Infections
Lorenzo Drago L et al.

Prospective cohort study

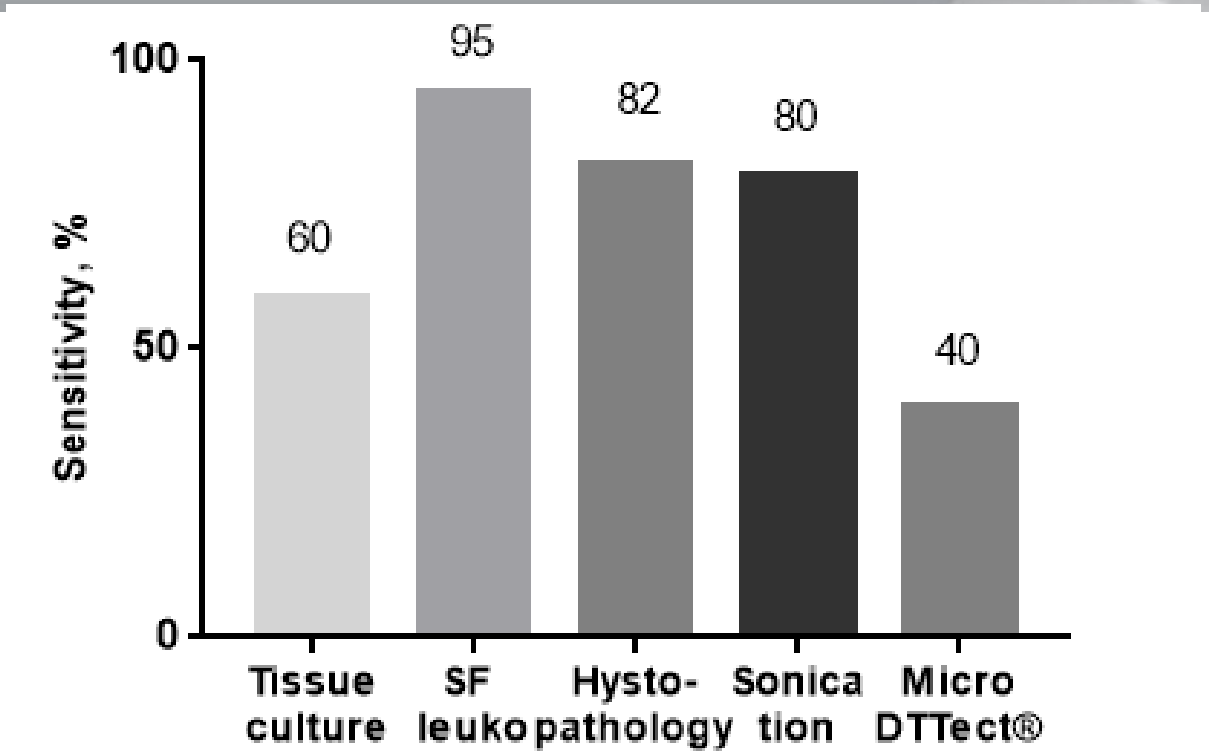
• to assessed performance of **DTT-based method (MicroDTTect®)** for biofilm removal from explanted prosthesis

93 Patients

102 Patients

	MicroDTTect®		Sonication	
	AF (n=58)	PJI (n=35)	AF (n=65)	PJI (n=37)
Age, median (range), yrs	70 (19-92)	69 (31-93)	68 (46-93)	73 (46-89)
Male gender, no. (%)	31 (53)	17 (49)	29 (45)	17 (46)
Affected joint, no. (%) **- knee** **- hip**	 17 (29) 41 (71)	 16 (46) 19 (54)	 36 (55) 29 (45)	 17 (46) 20 (54)

Results

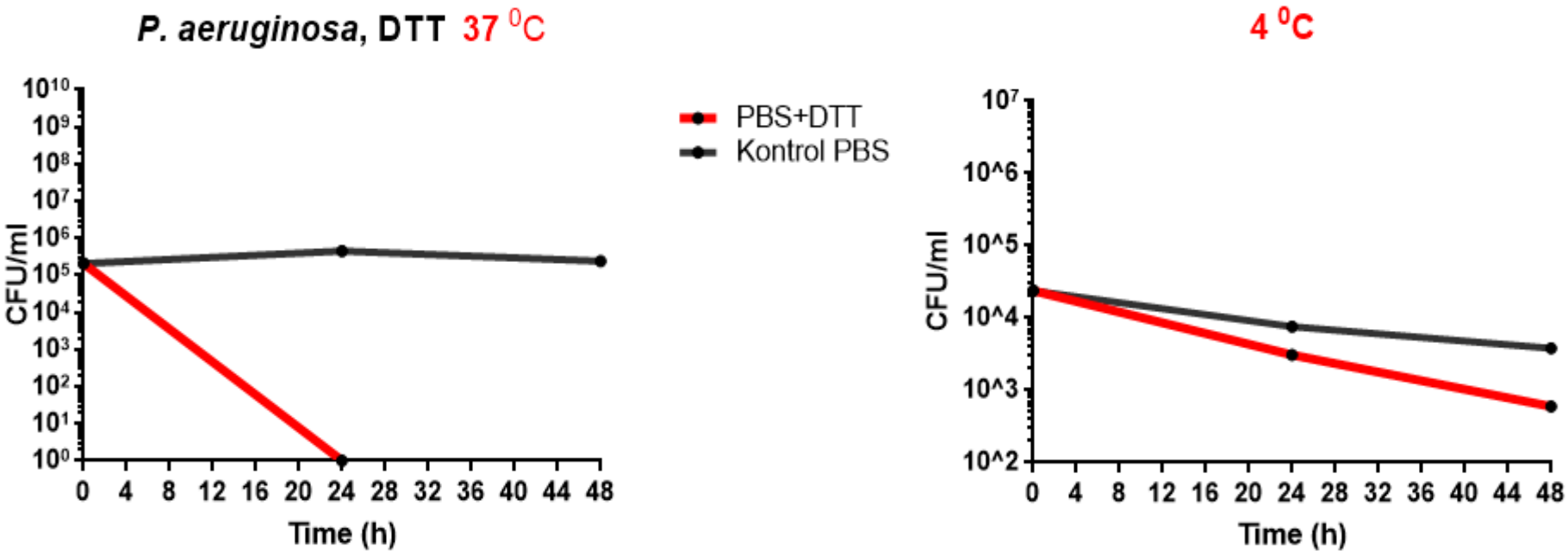


MicroDTTect® shows the lowest sensitivity for diagnosis of PJI in contrast with currently used diagnostic tests ($p<0.01$).

Sonication is superior to chemical method MicroDTTect® for the diagnosis of prosthetic joint infection ($p<0.01$).

	microDTTect	Tissue and/or SF culture
1	0	*MRSE*
2	0	*MRSA*
3	0	*C. perfringens*
4	0	*C. albicans*
5	0	*E. faecalis*
6	0	*MSSA*
7	0	*MRSE*
8	0	*MSSA*
9	0	*C. albicans*
10	0	*S. agalactiae*
11	*E. faecalis*	*E. faecalis/S. aureus*
12	*MSSE/Streptococcus mitis/oralis*	*MSSE*
13	*C. innocuum*	0
14	*MRSE*	0
15	*P. acnes*	0

Viability of bacteria in presence of DTT



DTT 1 g/L

Conclusions

- **Chemical methods** are not applicable in the routine microbiological diagnosis for biofilm detection

Conclusion

Sonication of the explanted prosthesis is the most sensitive tool in the intraoperative setting.

Thank you for your attention!

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