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DETECTION AND DESTRUCTION OF RESIDUAL DNA ON SURGICAL STEEL

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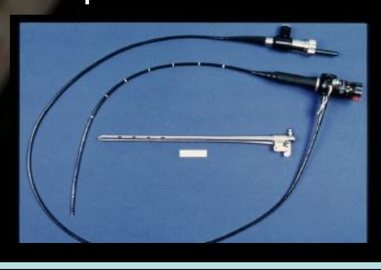
WHY?

Polymerase chain reaction (PCR) is being used increasingly in the field of clinical microbiology. One of the methods' advantages is the extreme sensitivity, but this can also be a problem, because even trace contamination with DNA can lead to erroneous diagnosis. This has been the case previously where carry-over of residual microorganisms from improperly cleansed bronchoscopes lead to false-positive PCR results. Current autoclave procedures are known to be adequate for killing of microorganisms, but it is not known whether the routines are sufficient for removal/inactivation of DNA. With molecular biology-based methods for diagnosis it could proove necessary to combine the autoclave step with a DNA removal/ inactivation method. Unfortunately, current strategies for removal/destruction of DNA from surfaces are hazardous, corrosive or expensive.

The aim of the current study was therefore to:

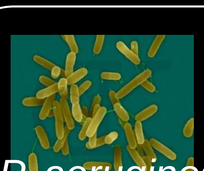
1) Develop a non-corrosive, non-hazardous, inexpensive method for making residual DNA non-amplifiable,
2) Devise a protocol for detection of residual DNA on surgical steel instruments
3) Determine whether residual DNA was present after sterilization routines at Aalborg University Hospital.



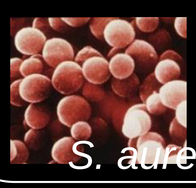
HOW?

Gefrides 2009: *"2 h of autoclave treatment will eliminate nanogram quantities of DNA from laboratory consumables"*

IN VITRO TEST



P. aeruginosa



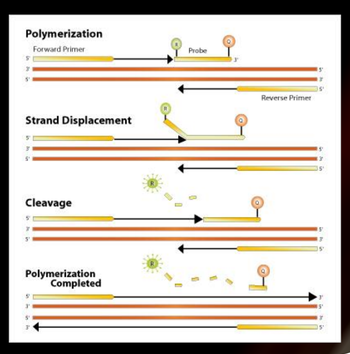
S. aureus

Autoclave step

a) Duration: 0 & 60 & 120 min
b) Temperature: 125 & 139 °C

DNA isolation and quantification

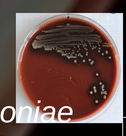
Sonication → soil kit
TaqMAN qPCR



CLIN. SIMUL.

P. aeruginosa

S. aureus



K. pneumoniae (thermotolerant)

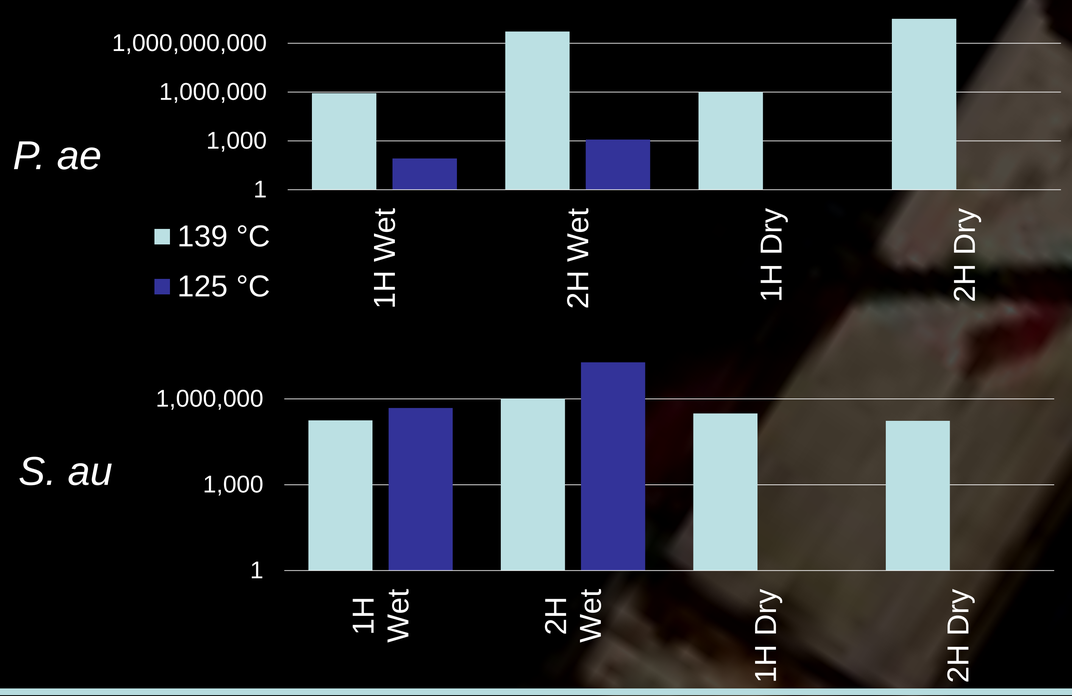
Wash 100 min
max temp 100°C

Autoclave

a) 4 min,137°C
b) 120 min,134°C

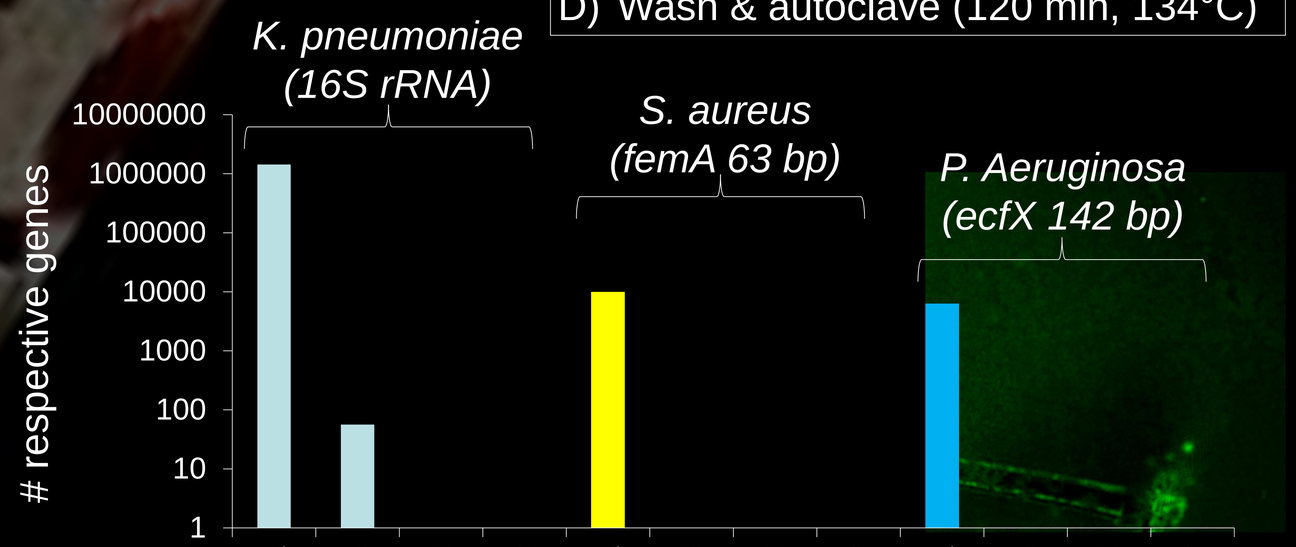
RESULTS

Dense inoculum:
10^9 cells/mL



Approximately 10^5 cells/scalpel

A) Control (no wash, no autoclave)
B) Wash (100 min, max temp 100°C)
C) Wash & autoclave (4 min, 137°C)
D) Wash & autoclave (120 min, 134°C)



CONCLUSION

The simulation at the CSR documented that the current disinfection routines are adequate. If more rigorous DNA-destruction is needed, prolonged autoclaving at 137°C for 120 min can be implemented to provide >10 mio fold reduction in the amount of residual amplifiable DNA.

REFS

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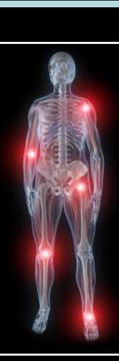

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