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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 prove 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:

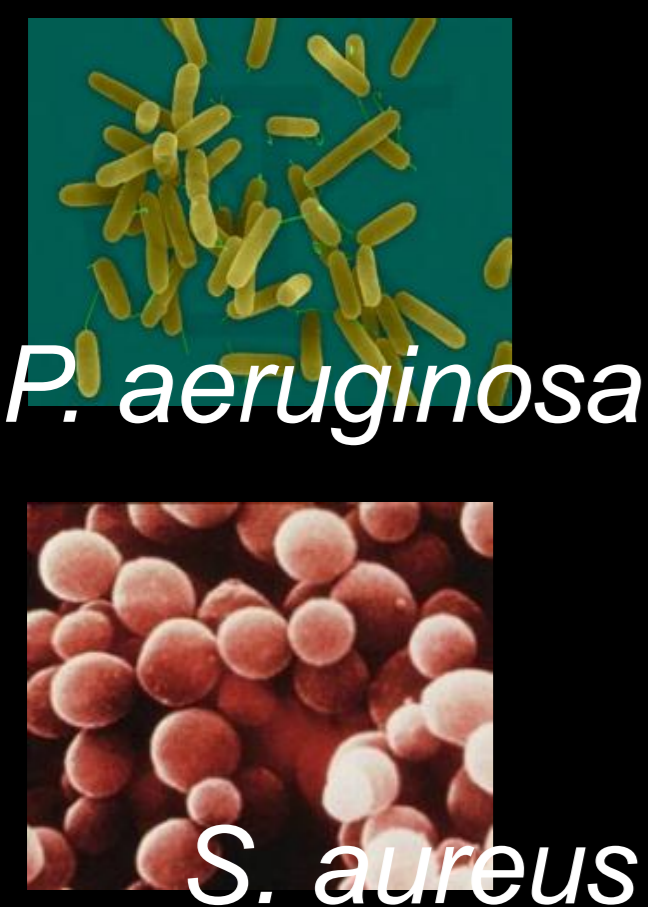
- 1) Develop a non-corrosive, non-hazardous, inexpensive method for making residual DNA non-amplifiable,
- 2) Devise a protocol for detection of residual DNA on surgical steel instruments
- 3) Determine whether residual DNA was present after sterilization routines at Aalborg University Hospital.



## HOW?

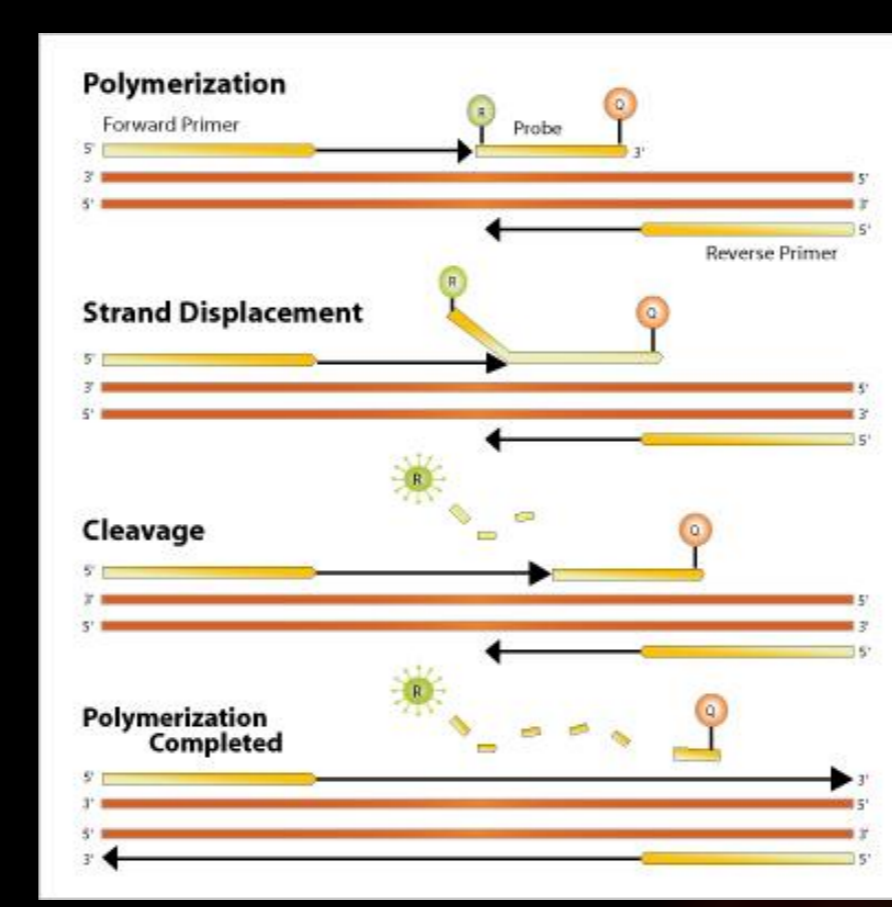
Gefrides 2009: "2 h of autoclave treatment will eliminate nanogram quantities of DNA from laboratory consumables"

IN VITRO TEST



**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)

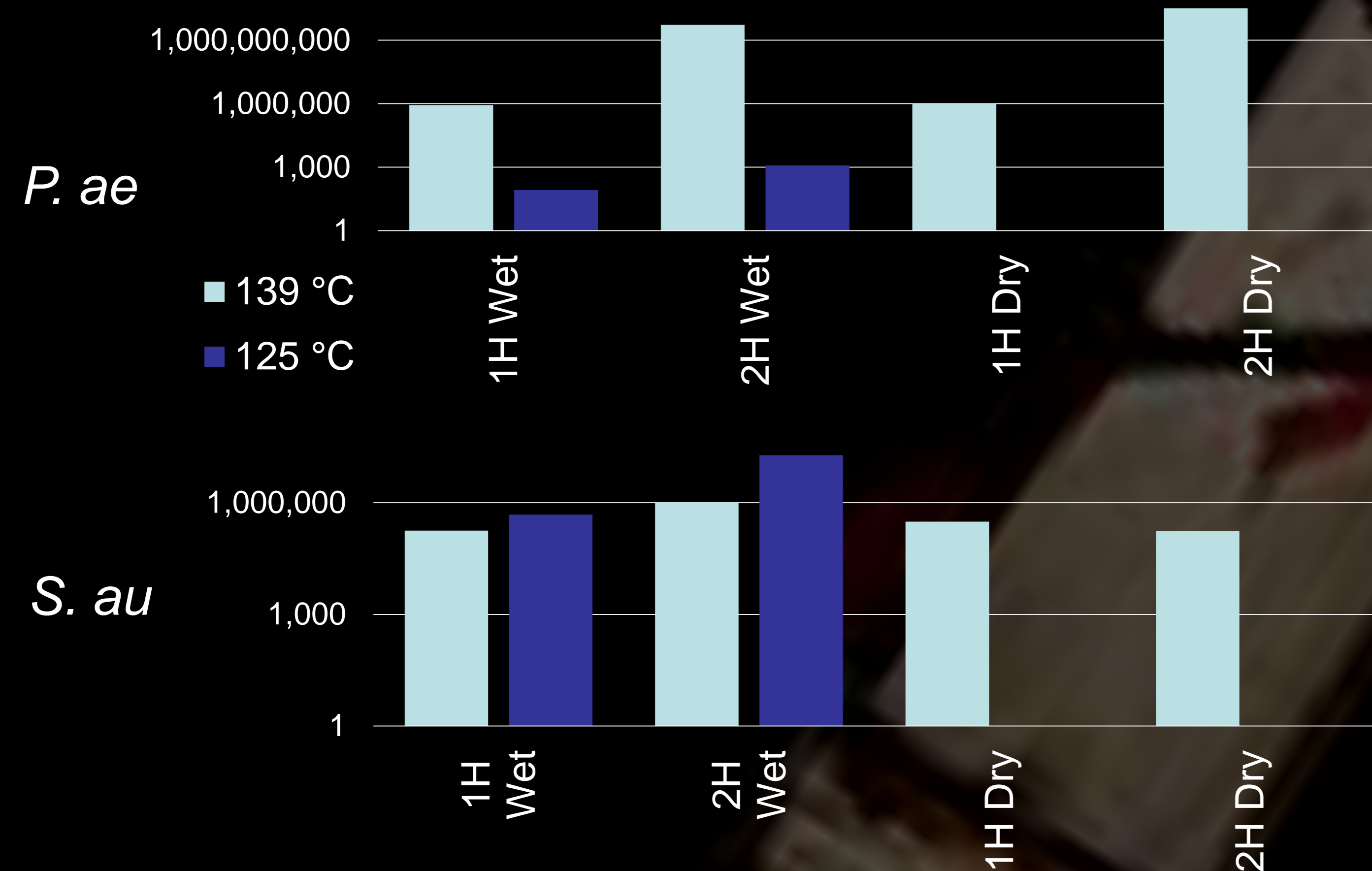


**Autoclave**

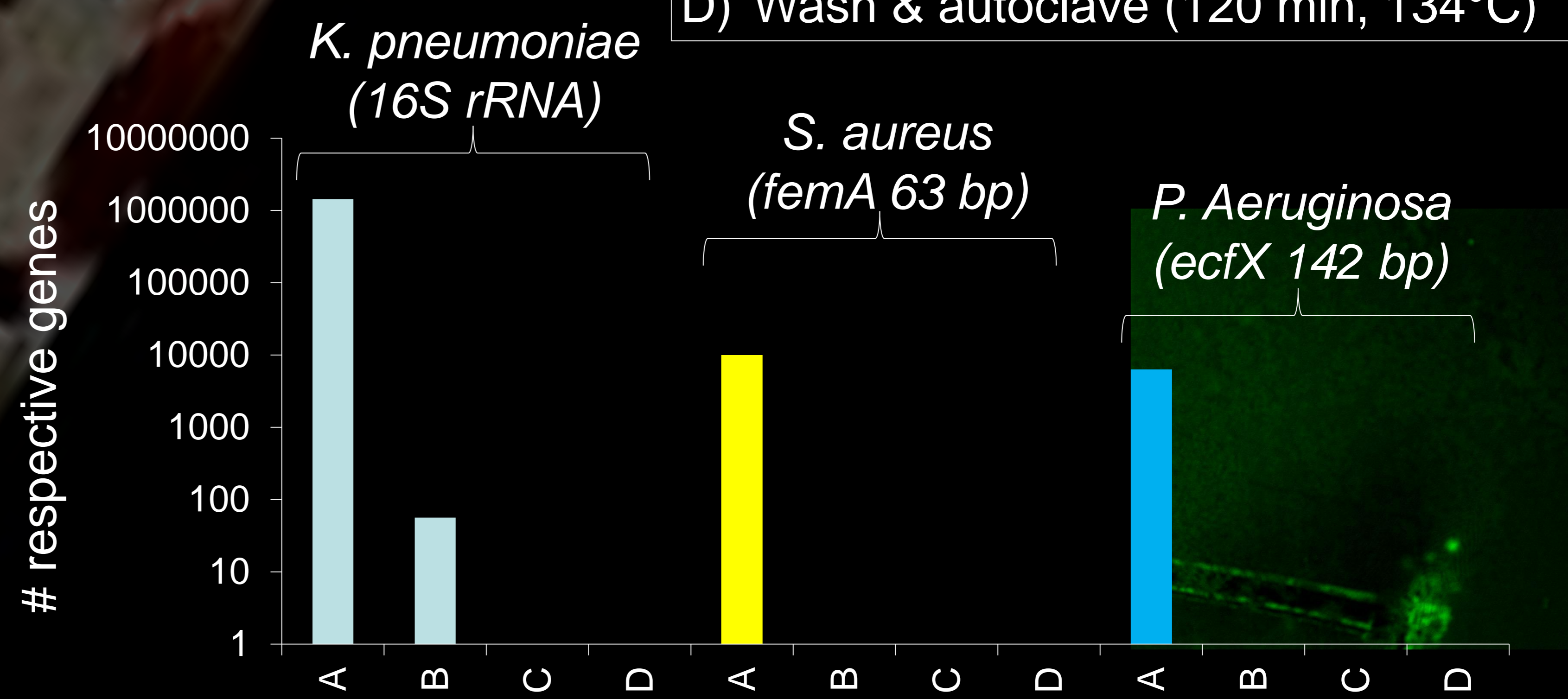
- a) 4 min, 137°C
- b) 120 min, 134°C

## 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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