

PRACTICAL CHALLENGES IN TODAY'S AND TOMORROW'S BCG PRODUCTION

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PRACTICAL CHALLENGES

- ❖ Culture principles
- ❖ Factors that influences Manufacturing
- ❖ Factors that influences Quality Control
- ❖ GMP issues



CULTURE PROPAGATION

- ❖ Propagation techniques
 - Surface culture
 - Submerged culture

Shift from submerged to surface culture technique has been related to increased adverse reactions (osteitis) in the Swedish strain

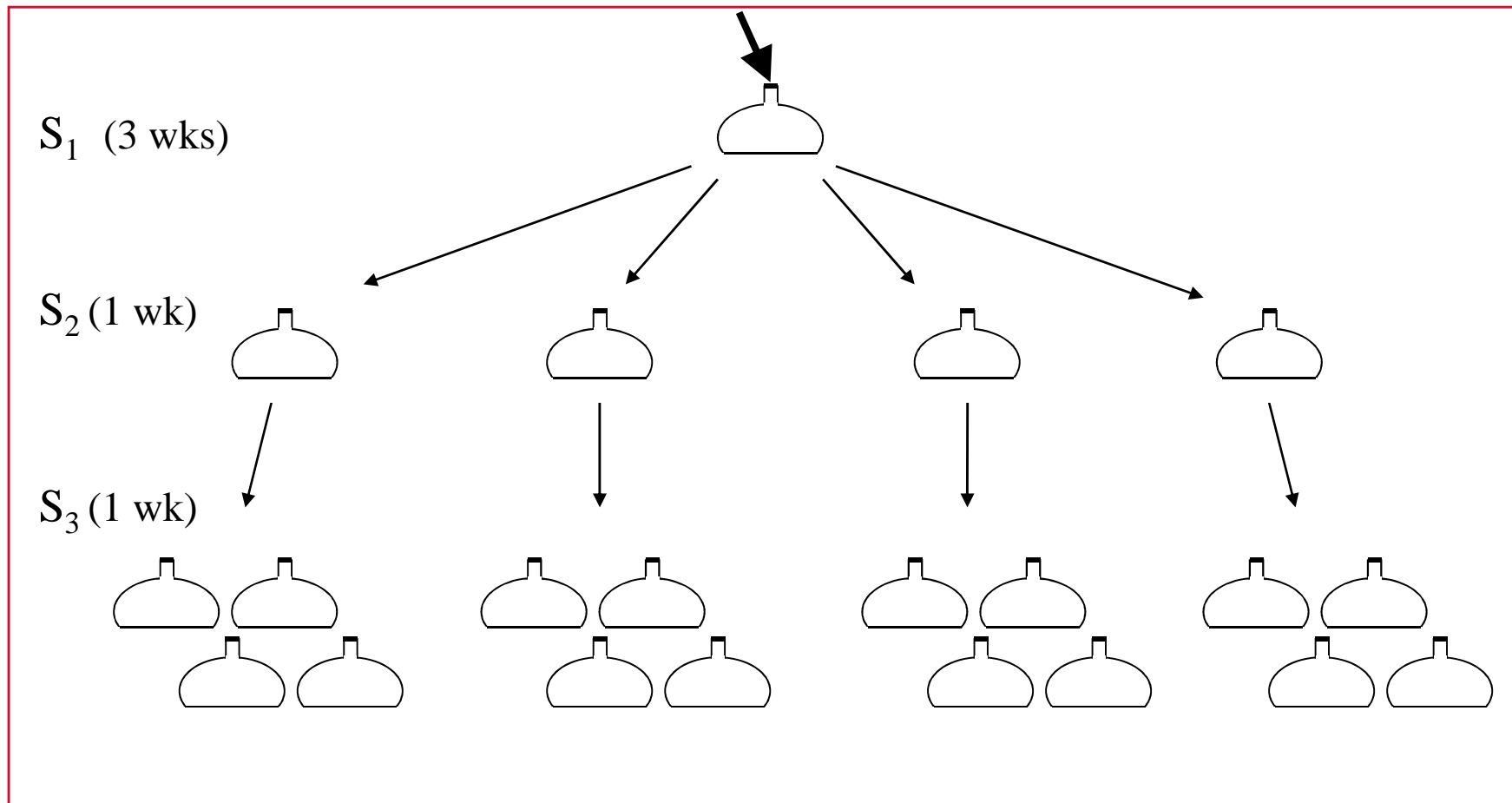
Shift from surface to submerged culture resulted in decreased tuberculin reactivity for the Glaxo 1077 strain



CULTURE PROPAGATION

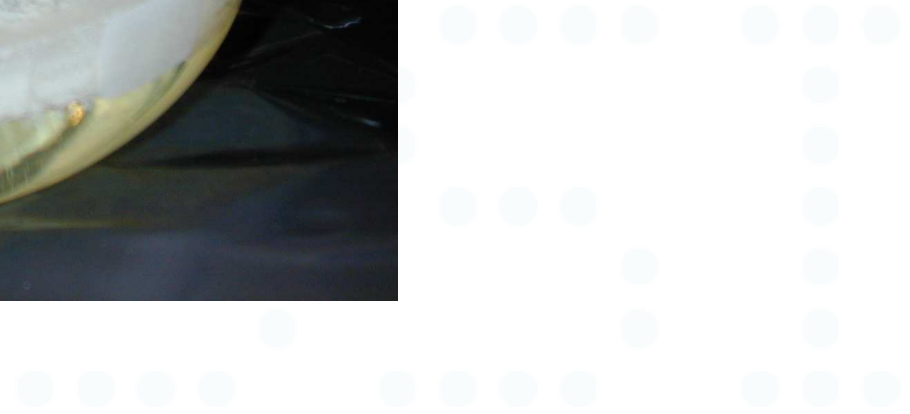
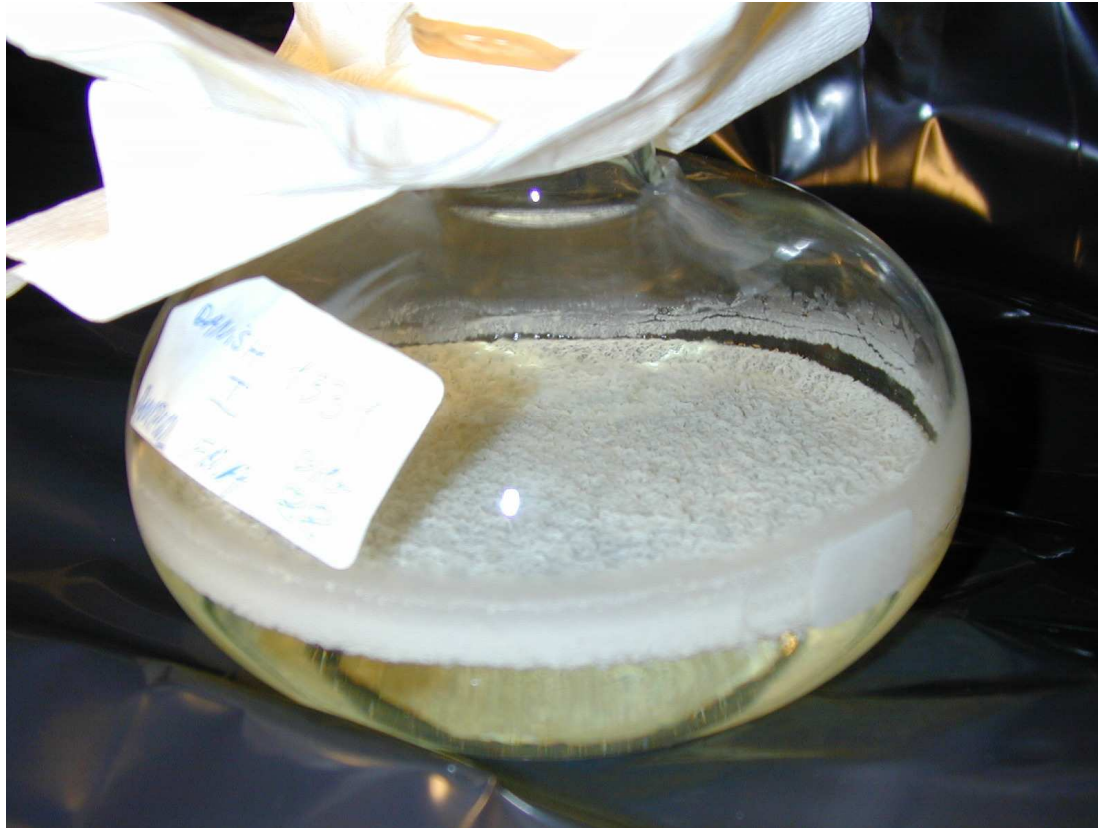
- ❖ Surface culture, Danish 1331

BCG seed-lot spread on Sauton medium

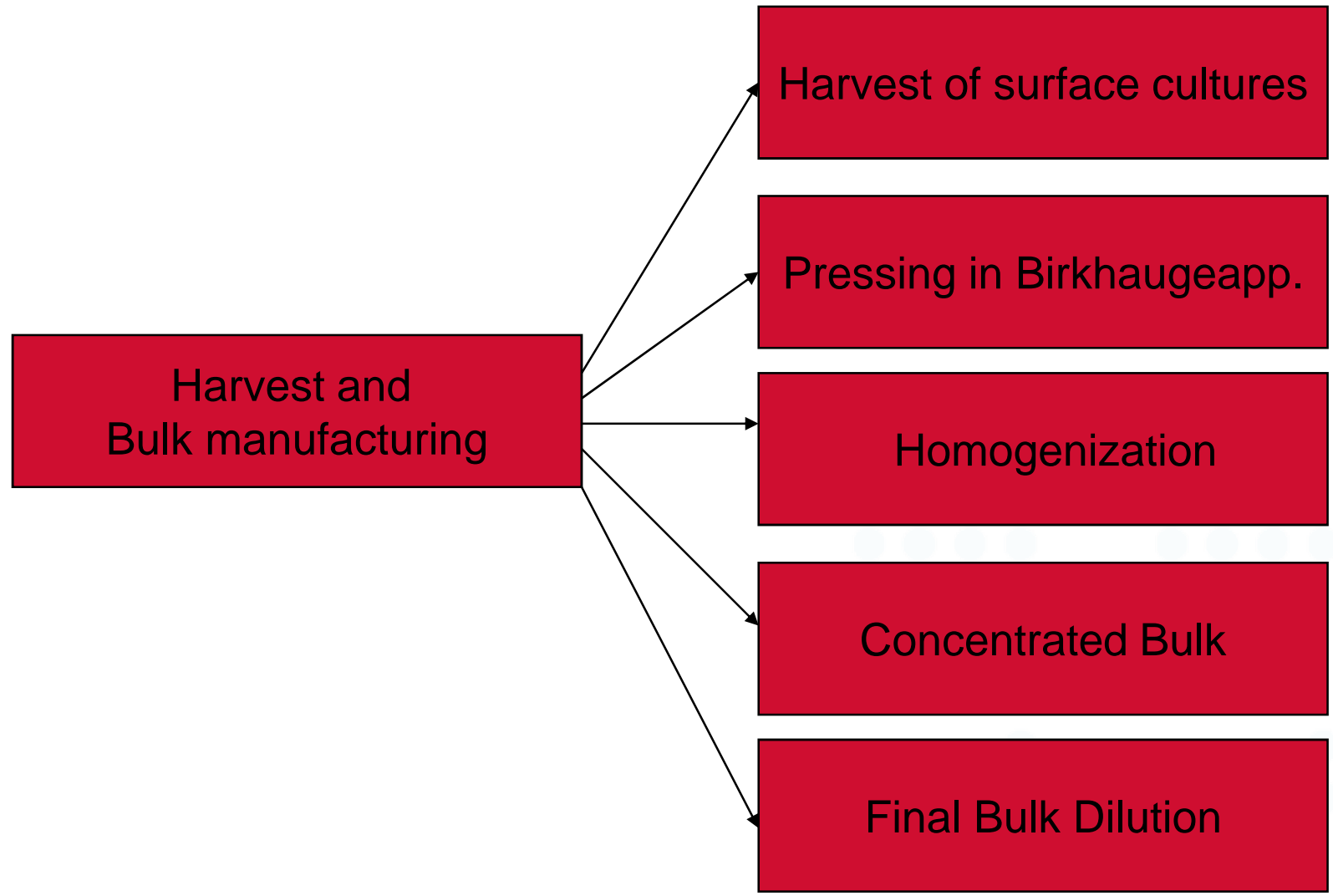


CULTURE PROPAGATION

- ❖ Danish 1331 surface culture



MANUFACTURING STEPS SSI



SOURCES OF VARIATION IN PROPAGATION

- ❖ Factors important to achieve a consistent product

Temperature

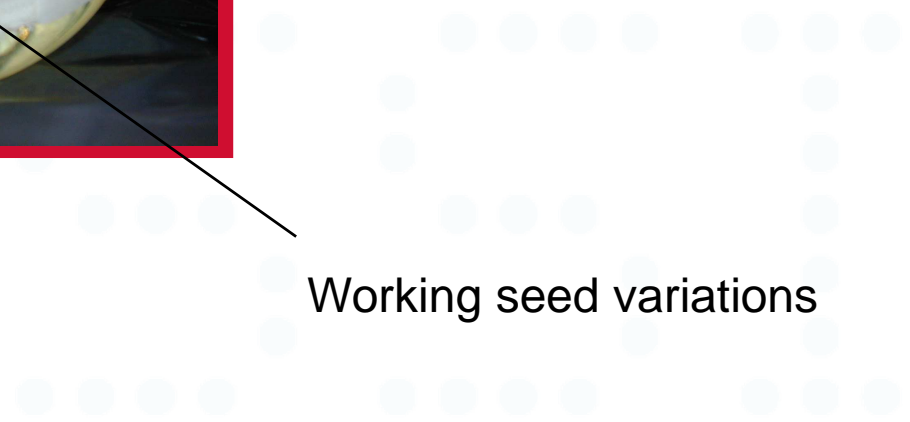
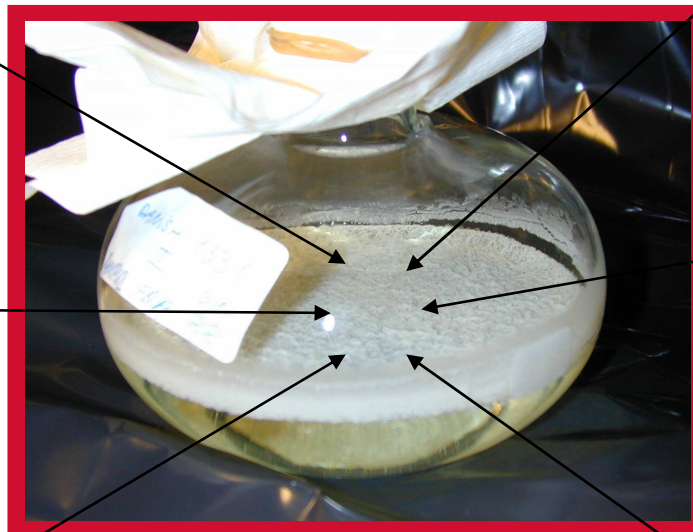
Growth medium variations

Humidity

Transfer techniques

Transfer times

Working seed variations



MEDIUM CONSISTENCY

❖ Trace minerals

- Sauton has traditionally been based on tap water
 - Dedicated tap in the manufacturing facility was the only source.
Plumbing work spoiled the source.
 - New source 30 km away in a small ground water facility



- Batch shifts in Citric acid changed growth performance

- Trace element analyses of tap water, Sauton medium and Citric acid

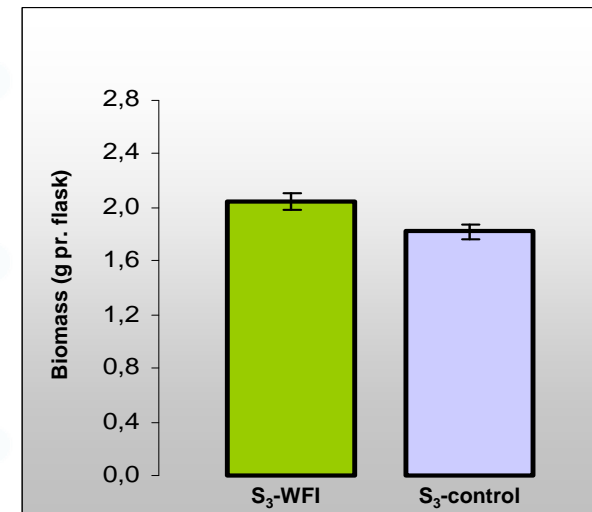
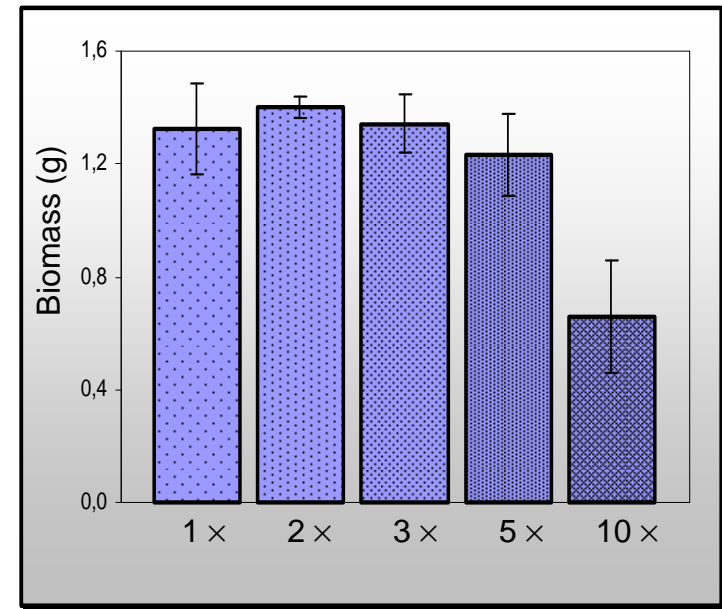
- 6 trace elements were identified
- Range of content 20-800 $\mu\text{g/mL}$



MEDIUM CONSISTENCY - WFI SAUTON

❖ WFI based Sauton medium

- Vendors of pure chemicals were tested in pilot scale
- Different levels of mineral addition was tested to check robustness
- Consistency in manufacturing, stability, reactogenicity investigated
- WFI based sauton implemented 2009
- Every new batch of raw material is checked for suitability
- Consistent growth capacity of Sauton enables consistent growth pattern and harvest conditions



❖ Raw materials for Sauton

- Glycerol oxidation to aldehydes slow down or prevent growth
 - Storage conditions
 - Container
 - Age
- Citrate
 - Different levels of trace element contamination

❖ Medium capacity

- When is a limiting component exhausted?

❖ Influence of Medium variations on product quality

- Growth rate
 - Culture growth phase at fixed transfer times
 - Initial growth rate from proceeding passage
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MEDIUM CONSISTENCY

- ❖ Culture medium influences the vaccine properties in mice
 - Venkataswamy et al.; Vaccine 30 (2012)
- ❖ Control over medium consistency is crucial for a consistent product quality
- ❖ Medium consistency are one of the largest GMP issues.
- ❖ Medium changes are regulatory issues
 - Even if they are “just” standardisations
 - Optimal medium composition is not an option



TRANSFER AND HARVEST CONDITIONS

- ❖ Transfer and harvest conditions of BCG culture is of outmost importance for a consistent product
 - Transfer amount
 - Transfer time
 - Medium capacity
 - Supported growth rate
- Properties of harvested cultures important for:
 - Resistance to homogenization
 - Survival rate after freeze-drying
 - CFU of final vaccine
 - Growth stage of freeze-dried vaccine



Passage transfer

- ❖ Consistent product may not appear consistent in QC
 - Variations in media for CFU counts
 - Source of eggs for L-J medium
 - Batch size of L-J batch
 - Temperature profile of ovens

 - Techniques for performing CFU test on L-J medium
 - Trained OMCLs obtain different results
 - Parallel testing of exchanged samples perform differently
 - Parallel testing is a requirement for release in some countries

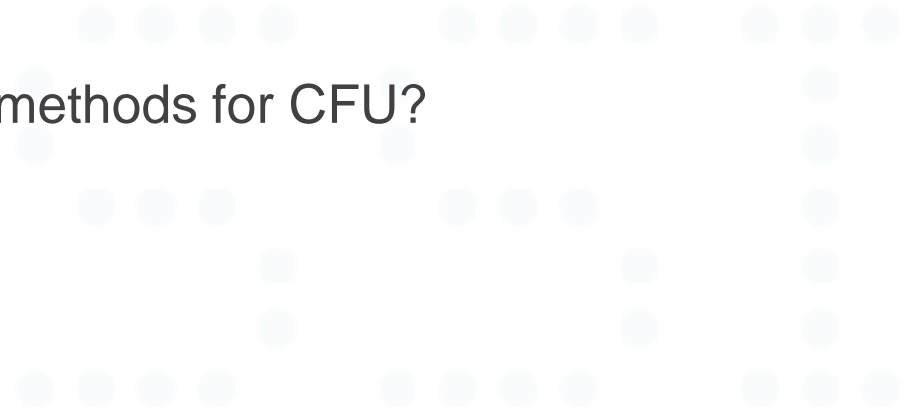
 - Efforts to obtain reproducible results
 - Alternative methods
 - ATP – influenced by growth medium conditions in relation to L-J
 - Defined medium e.g. 7H10 – level change
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CFU TEST AND LIMITS

- ❖ CFU limits for current BCG vaccines
 - Empirical
 - Limits are linked to chosen substrate

- ❖ Control of CFU methods
 - Stable reference preparation
 - For tracking medium performance
 - For tracking testing technique

- ❖ New rapid testing methods
 - Can they ever correlate to existing methods for CFU?
 - May be used for trouble shooting



CURRENT AND FUTURE CHALLENGES

- ❖ Standardization of production and QC methods
 - Slow growth
 - Propagation strategy
 - Medium variations
 - Process validation

 - Passage limitations
 - How many passages can be allowed
 - Japanese and Danish strains have been followed for 20
 - No change in mRNA expression profiles
 - Is this method satisfactory

 - Characterisation
 - Which methods are applicable
 - New analytical methods will most likely detect genetic variations
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- ❖ BCG production were not established in a GMP era
- ❖ Efforts to standardize and improve existing products to GMP
 - Regulatory difficult
 - Clinical effects are hard do predict and test
- ❖ Characterisation expectations
 - Genetic and biochemical characterisation expectations for new vaccines may not make sense for existing BCG products
- ❖ Testing methods are subject to as much variation as the manufacturing it self
 - New more standardised methods correlation to existing methods are influenced by even small variations in manufacturing

CONCLUSION

- BCG vaccine and GMP are not natural companions



REFERENCES

- ❖ In vitro culture medium influences the vaccine efficacy of Mycobacterium bovis BCG; Venkataswamy et al.; Vaccine 30 (2012) 1038 – 1049
- ❖ Letter to the editor - Swedish strain; A Lind; Tubercle 64 iss. 3 (1983)
- ❖ Glaxo: Tuberculin sensitivity and skin lesions in children after vaccination with 11 different BCG vaccines; Vallishayee et al.; Bull. WHO 1974, 51, 489-494
- ❖ Assay in man of different BCG products; Nyboe et al.; Bull. WHO 1966, 35, 645-650.

