Spore Lot Consistency: Points to Ponder

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NIAID Aerosol Challenge Workshop

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Considerations

- Challenge Material
- Standardized Protocols/Procedures
- Media
- Characterizations
- Purity
- Viability
- Storage Conditions

Challenge Material

- Will NIAID provide seed stock of challenge material or will they provide challenge material “ready to use”
- Protocols for harvest and purification
- Protocols to characterize lots
- Qualification standards

Media

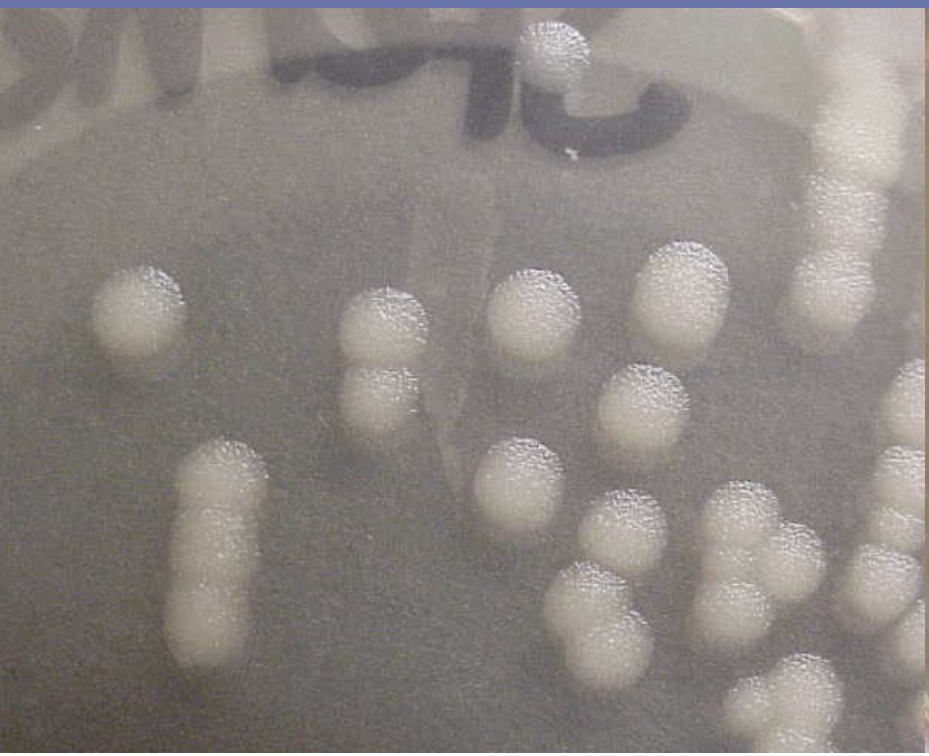
- Agar vs. Broth
  - agar harvest more time consuming; total percent sporulation may be lower
  - differences in virulence
  - LD_{50} values higher when spores grown on agar

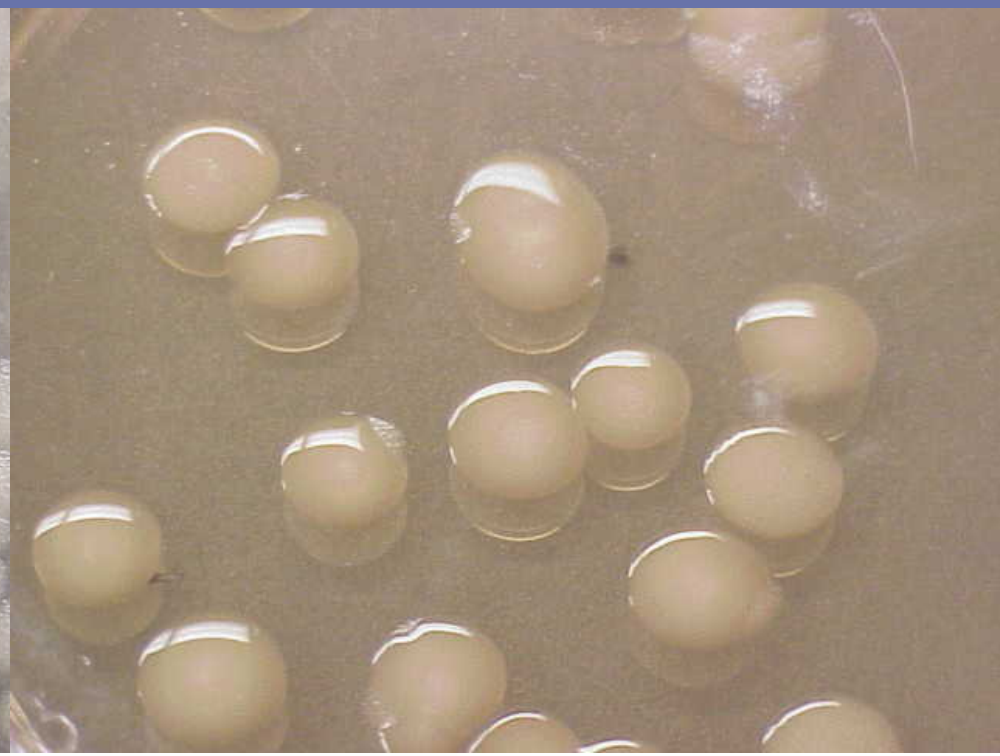
	LD_{50}-blood agar	**LD_{50}-L&D**
Vollum 1B	2501	307
Ames	488	175
Texas-2	740	519

Ivins & Fellows, unpublished data

Characterizations

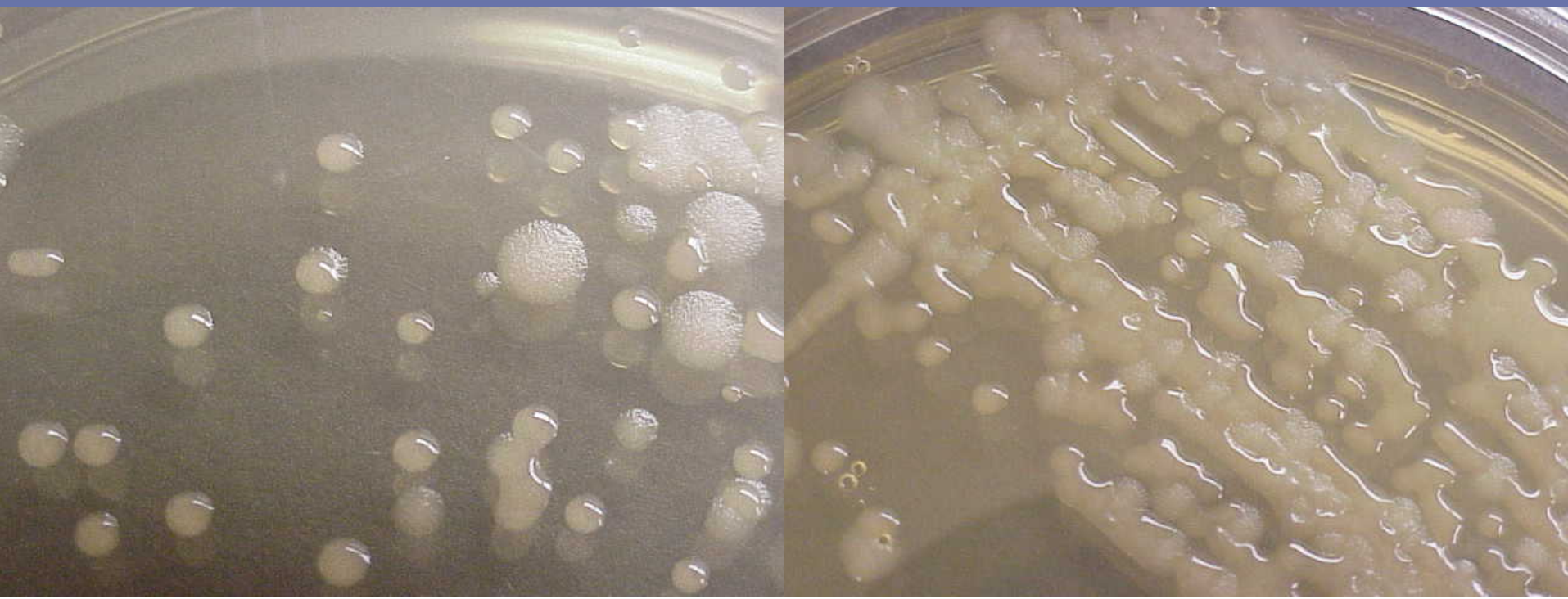
- Genetic Characterizations
  - genotypic analysis
- Phenotypic Characterizations
  - pX01 toxin gene expression
    - Protein gel analysis to identify PA, LF, EF
    - Biological activity - rat lethality; rabbit/guinea pig skin test
  - pX02 capsule gene expression
    - mucoid phenotype



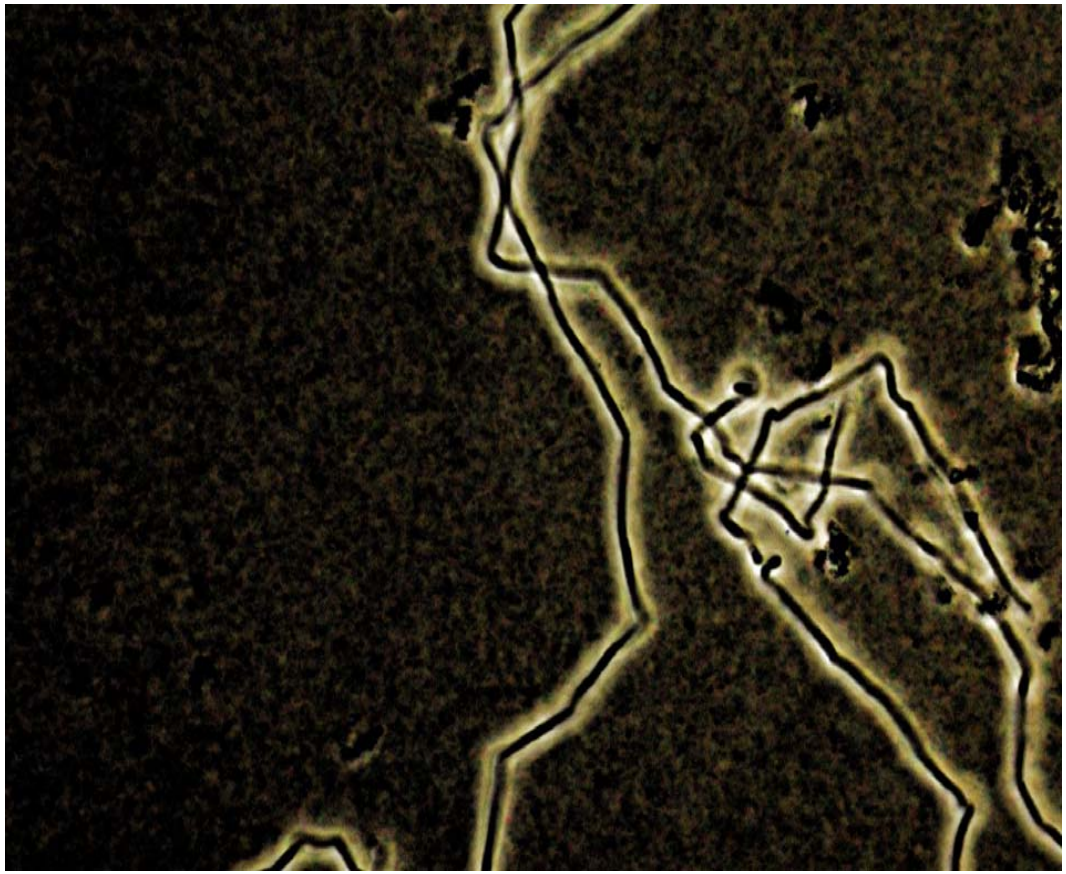


Sterne - non-encapsulated

Ames - encapsulated



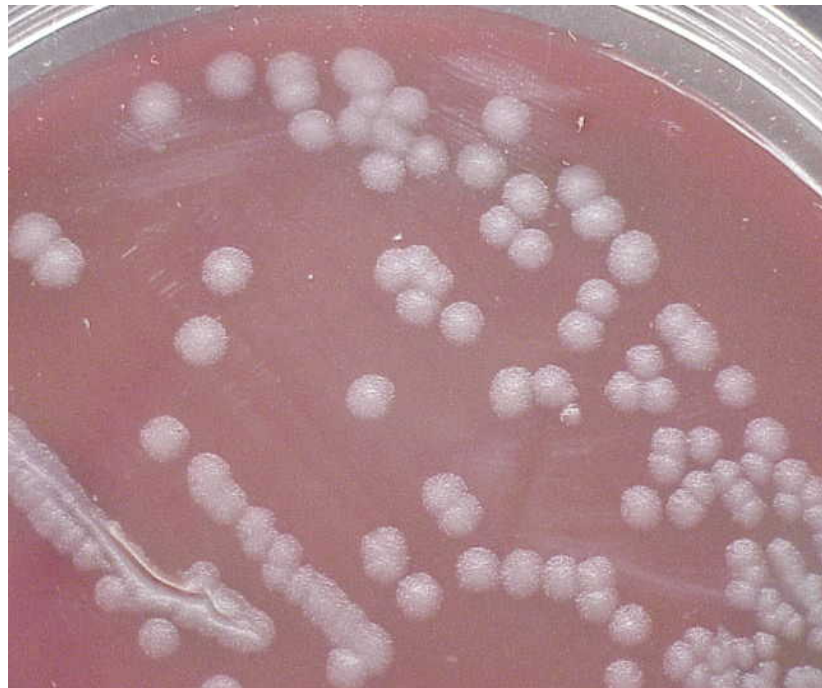
Mixture of encapsulated and non-encapsulated colonies - not Ames, however, this is indicative of loss of pX02 and therefore loss in virulence



India Ink Staining identifies the presence of capsule

Purity

- Colony morphology
  - free of contaminants
- Hypaque purification
  - density gradient that separates spores from vegetative cells & debris
- Microscopic evaluation
    - percent refractility
    - Malachite Green staining



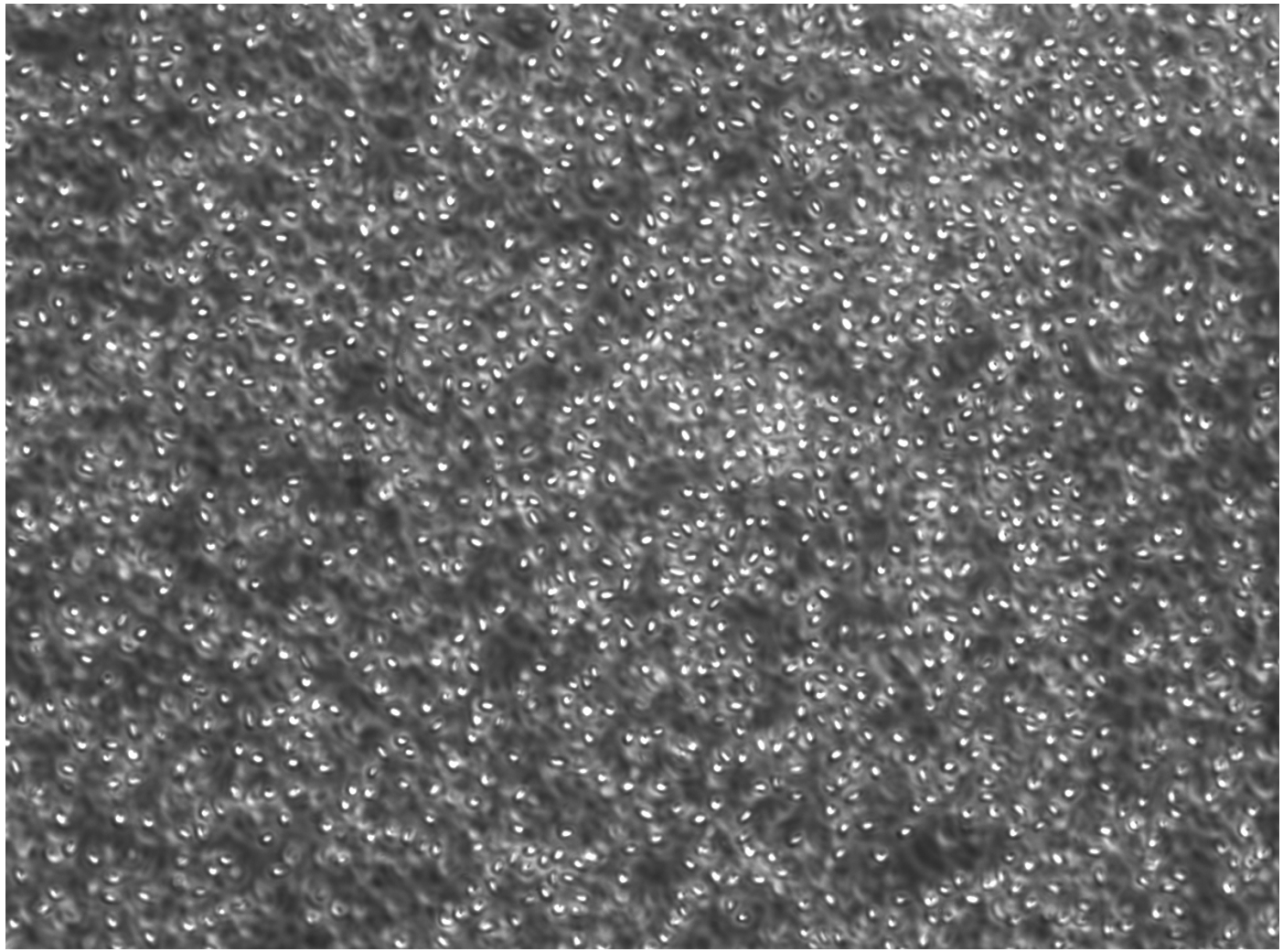
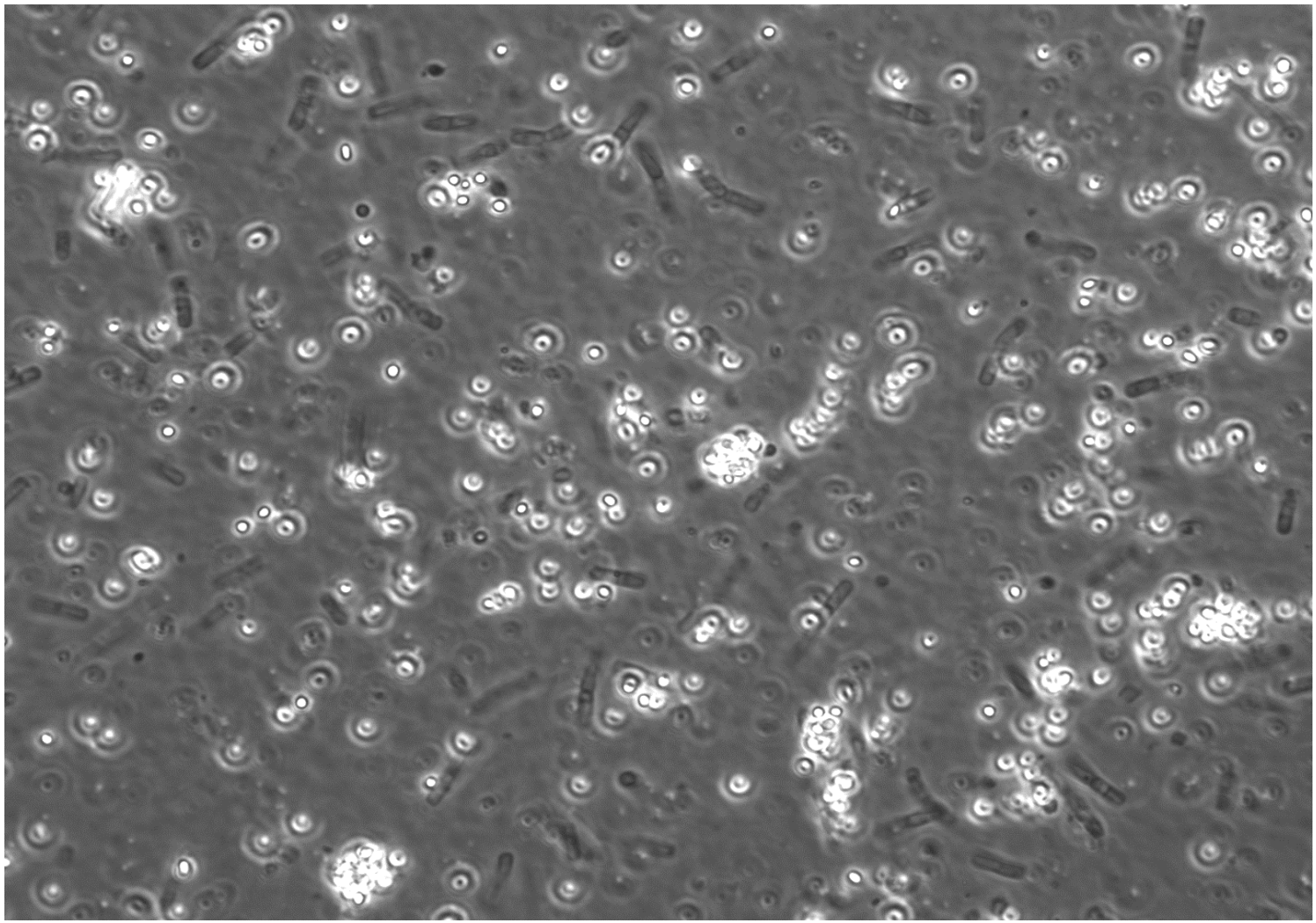


Photo courtesy of Joel Bozue



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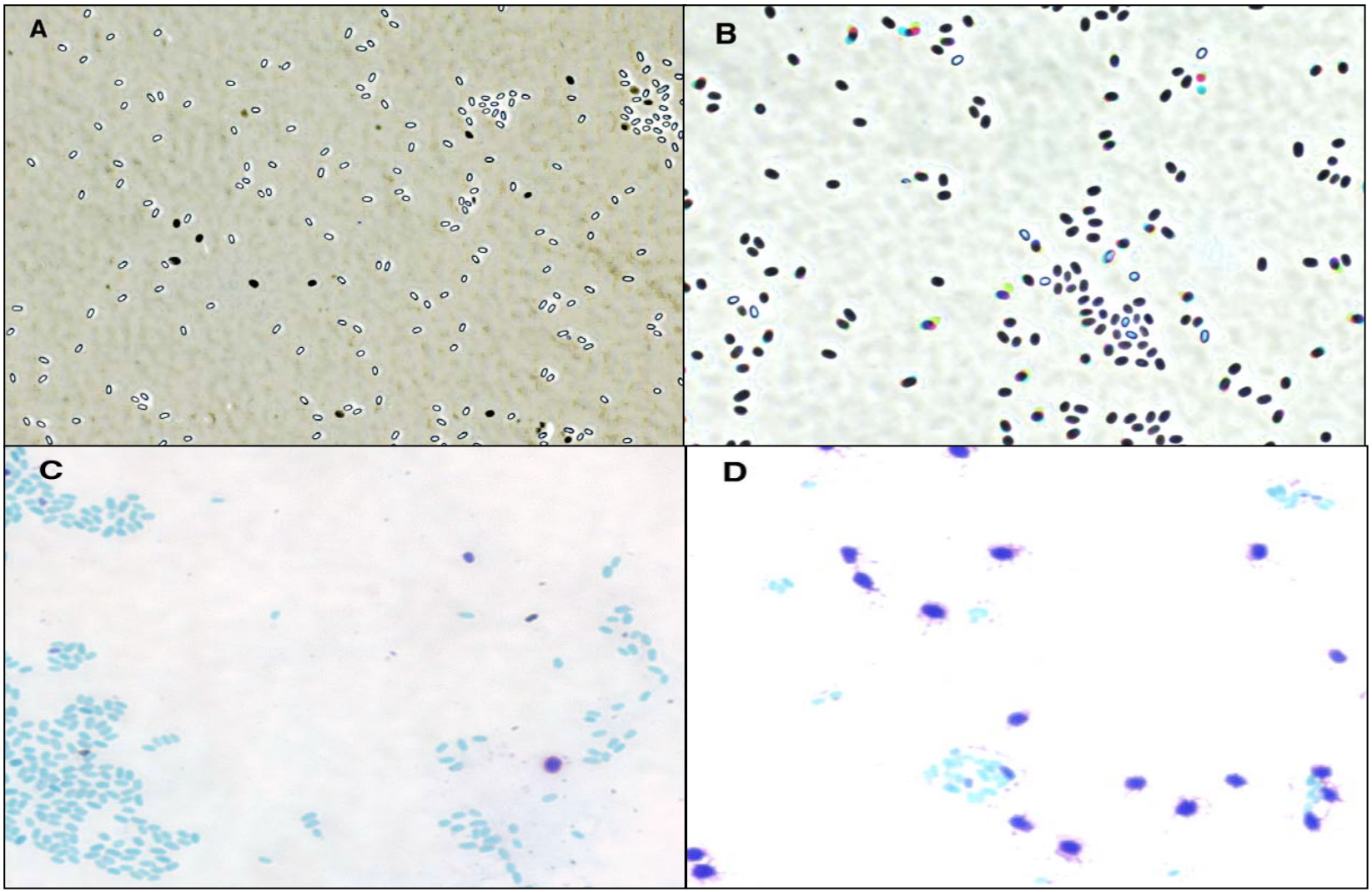

Photos courtesy of Sue Welkos

Viability

- Viable counts
  - periodic plate counts
- LD_{50} Determinations
  - initial determination
  - periodic
    - yearly
    - lot to lot bridging

Storage Conditions

- Aliquots of known concentration can be stored at -70^{o}C with glycerol
- Batches can be stored in 1% phenol at 4^{o}C
  - highly concentrated; not recommended for “working stock” concentrations lower than 10^{7}
  - type of container important
    - glass and polycarbonate appropriate storage

Challenge

- Spores must be prepared at the appropriate concentration required for challenge as well as taking into account “spray efficiency factor”

- Are spores heat shocked just prior to challenge
  - synchronizes spores for germination
  - get rid of any vegetative cells

Conclusions

Protocols and standards should be developed that consider:

- Procedures for spore harvest and purification
- Genotypic & Phenotypic charcteristics
- Viability and LD_{50} determinations
- Storage conditions
- Procedures for challenge