

Johnin PPD Production

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Johnin PPD Project History

- 1998-2000: NVSL/ISU collaborative production of 4 batches of Johnin PPD
- TCA precipitation method
- Evaluation of batches has continued to show variable results
 - Protein analysis
 - Cattle and sheep studies



Johnin PPD Project History

- 2005 USAHA Johnine's Committee
 - USAHA Resolution No. 20
 - NVSL develop a protocol for the production of a consistent, quality Johnin PPD
 - NVSL produce Johnin PPD for research evaluation
 - Potential use for further research
 - Skin test product
 - INF- γ protein



Original Concerns

- ❑ Large quantities of media made in smaller multiple batches introduce a new variable
- ❑ Additives to the media
- ❑ Strange proteins that could only be introduced through autoclaving
- ❑ Different incubation periods affecting the protein content and potency of final product
- ❑ Heat kill versus sterile filtration of culture

Media: Modified Reids w/ Mycobactin and Sodium Pyruvate

- ❑ Comparison study: Filtered Media versus Conventional Autoclaved Media.
- ❑ 35 Povitsky bottles - 500 ml of media per bottle.
- ❑ Media from two separate autoclave sterilizations distributed equally between the methods tested.

Two Methods of PPD Production

- Group A - Povitsky bottles are autoclaved at 121° C for 30 minutes and allowed to cool overnight. The contents are then filtered through 85 mesh nylon and gauze supported by stainless steel mesh.
- Group B- Povitsky bottles are filtered using commercial filters. The filtrate is autoclaved at 121° C for 30 minutes and allowed to cool overnight.

Number of Povitsky Bottles per Method

Method	Number of Povitsky Bottles	Number of Individual Bottles
Group A	10	5
Group B	10	5
Total Number of Bottles	20	10



QA Procedures

- ❑ Streak blood agar plate from each Povitsky bottle to check individual bottle sterility
- ❑ Un-inoculated bottle is incubated to check for the sterility maintenance throughout entire incubation period
- ❑ Send culture to the Mycobacteria section for confirmation of identification



Procedure for Individual Bottles

- ❑ Proteins precipitated with trichloroacetic acid (TCA).
- ❑ Two washes with 1% TCA
- ❑ Wash with 10% NaCl.
- ❑ Submit to NADC for a protein standardization

Procedure for Large Batches

- ❑ Proteins precipitated with trichloroacetic acid (TCA).
- ❑ Two washes with 1% TCA.
- ❑ Wash with 10% NaCl.
- ❑ Check protein level using micro-Kjeldahl.
- ❑ Standardize PPD to 1 mg /1 ml with buffer.

Evaluation of Individual Povitsky Bottle Products

- ❑ Test using the IFN- γ assay using NADC positive Johne's cattle
- ❑ Use Lot 1 as the control to compare results
- ❑ Complete protein evaluation of all ten products by NADC
- ❑ Compare results between bottles and methods to determine most efficacious method

Evaluation of the Two Large Batches

- Data collection – NVSL functions as the data and information central clearinghouse
- Standard – Johnin PPD Lot 1
- Field studies – How many animals? Testing personnel or team effort? Known negative herds? Open to proposals.
- Guinea pigs – How many animals? Funding? Testing personnel? Or will the IFN- γ Assay suffice? Do the CFR regulations apply here?



Evaluation of the Two Large Batches

- ❑ Protein – Micro-Kjeldahl vs. iTraq Labeling
- ❑ Species – Cattle? Sheep? How many products per animal (1-3)?
- ❑ Evaluation(s) – Who will the make the decision?



Possibilities for Further Study

- Evaluate how different time spans for organism growth affect final product.
- Evaluate product performance in other species.



Thank You

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