FINAL SCRIPT: APPROVED FOR FILMING

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Title: Separation and Fractionation of Cell Wall and Cell Membrane Proteins from *Mycobacterium tuberculosis* for Downstream Protein Analysis

Authors and Affiliations:
Anbarasu Deenadayalan[1], Madhavan Dhanapal[1], Uma Devi Ranganathan[1,2], Ramalingam Bethunaickan[1,2]

[1]Department of Immunology, National Institute for Research in Tuberculosis
[2]Faculty of Medical Research, Academy of Scientific and Innovative Research (AcSIR)

Corresponding Authors:
Ramalingam Bethunaickan (bramalingam@gmail.com, ramalingam.b@icmr.gov.in)
Email Addresses for All Authors:
Anbarasu Deenadayalan (harianbu1@gmail.com)
Madhavan Dhanapal (sakthimadhavan84@gmail.com)
Uma Devi Ranganathan (krumadevi@gmail.com)
Ramalingam Bethunaickan (bramalingam@gmail.com, ramalingam.b@icmr.gov.in)

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 12
Number of Shots: 22

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Ramalingam Bethunaickan:** Our research focuses on developing a streamlined method to separate insoluble cell wall and membrane proteins of *Mycobacterium tuberculosis* into simple fractions for direct proteomic and immunological assays.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.2*

What are the most recent developments in your field of research?

1.2. **Ramalingam Bethunaickan:** Advances in electrophoresis now allow complex proteomes to be separated into 2–4 protein fractions, facilitating efficient Mass Spec-based isolation for functional, proteomic, and immunological applications.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What are the current experimental challenges?

1.3. **Anbarasu Deenadayalan:** Key challenges include poor solubility of hydrophobic proteins—precipitating above 100 mg—and SDS-PAGE-induced denaturation, which hinders downstream applications needing native conformation, like enzyme activity assays.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.4. **Madhavan Dhanapal:** No current protocol systematically separates insoluble hydrophobic cell wall and membrane proteins into well defined, water-soluble fractions for direct proteomic and immunological analysis which is key to understanding function and guiding interventions.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the future?

1.5. **Uma Devi Ranganathan:** Future research will develop T cell antigen–based diagnostics, evaluating their sensitivity, specificity, and performance across TB populations to enhance early, accurate detection and support better disease control.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Scientific Advisory Committee and Ethical Committee at ICMR-NIRT

Protocol

Videographer's Note: Since both the articles #67680 & #67679 had similar shots, clapboard labelling is according to the shot numbers of #67679. Please have the final script of #67679 for referral and understanding.
Further notes to editor:

1. **#67680 includes a lot of footage from the shoot of article #67679.**
2. **For the protocol videos, all the natural light coming in either had green or blue coloured windows. Please balance the colour temperature closest to pure white, for the entire final video.**
3. **Panasonic LUT has been attached to the post shoot notes mail, if needed.**
4. **I have shot 2 variations - 1 landscape & 1 portrait each for headshots of the authors. Please use them interchangeably for both #67679 & #67680.**

2. Preparative Liquid-Phase Isoelectric Focusing of *Mycobacterium tuberculosis* Cell Fractions

Demonstrator: Madhavan Dhanapal

2.1. To begin, obtain the cell wall, cell membrane and cytosolic protein samples from whole cell lysate of *Mycobacterium tuberculosis* bacilli **[1].** Solubilize the cell wall and cell membrane proteins in isoelectric focusing buffer **[2-TXT]**.

2.1.1. WIDE: Talent holding labelled vials containing cell wall, cell membrane and cytosolic protein isolates.

2.1.2. Talent adding reagents into a tube containing membrane protein pellet and mixing the sample. **TXT: IEF buffer: 8 M urea, 1 mM DTT, 5% glycerol, 2% digitonin**
Videographer's Note: 2.1.2 has been labelled in the clapboard as 2.1.2 - 1

2.2. Add 2 percent ampholytes between pH ranges of 3 to 10 and 4 to 6, in a ratio of 1 to 4 **[1]**.

2.2.1. Talent pipetting ampholytes from labeled stock solutions and adding them into the solubilized protein mixture.

2.3. Now, fractionate the solubilized proteins in a liquid isoelectric focusing system maintained at 4 degrees Celsius with a cooling water bath **[1]**. Conduct isoelectric focusing separation following the manufacturer's instructions by applying a constant power of 12 watts **[2]**.

2.3.1. Talent loading the sample into the liquid IEF system connected to a water bath.
Videographer's Note: Shot 3.3.1 (From #67679)

2.3.2. Talent inputting 12 watts and monitoring the voltage increase until stabilization.
Videographer's Note: Shot 3.3.3 (From #67679)

2.4. Collect the individual isoelectric focusing fractions with a vacuum pump **[1].** Determine the pH values of each fraction **[2]**.

2.4.1. Talent drawing liquid fractions from the IEF apparatus using a vacuum pump.
Videographer's Note: Shot 3.4.1 (From #67679)

2.4.2. Talent testing the pH of the fractions on a pH paper or using a pH meter.
Videographer's Note: Shot 3.4.2 (From #67679)

2.5. Subject 50 micrograms of the separated fractions to SDS-PAGE and visualize the proteins using Coomassie Brilliant Blue or silver staining **[1-TXT]**.

Added shot: Talent loading aliquots on SDS-PAGE gels.
Videographer's Note: Shot 3.4.3 (From #67679)

2.5.1. Shot of separated fractions on a PAGE gel, post staining. **TXT: Store the separated IEF fractions in a - 80 °C freezer till further analysis**
Videographer's Note: Shot 2.7.4 (From #67679)

3. Preparative Two-Dimensional Electrophoresis and Protein Elution from Polyacrylamide Gels

Demonstrator: Anbarasu Deenadayalan

3.1. Start by mixing the IEF *(I-E-F)* separated cell wall and cell membrane fractions with SDS gel electrophoresis sample buffer **[1-TXT].** Heat the mixture at 95 degrees Celsius for 5 minutes **[2]**.

3.1.1. WIDE: Talent adding SDS-PAGE buffer to the sample tubes. **TXT: Use 6x SDS buffer**
Videographer's Note: Shot 4.1.1 (From #67679)

3.1.2. Talent placing the mixture in a heating block and setting temperature to 95 degrees Celsius.
Videographer's Note: Shot 4.1.2 (From #67679)

3.2. Separate the protein fractions in the second dimension using 16 by 20-centimeter polyacrylamide gels with a 12.5 percent resolving gel and a 4 percent stacking gel **[1]**. Then use a single 13-centimeter long sample well to load the sample onto the gel **[2]**.

3.2.1. Talent pouring stacking gel onto the semi-assembled gel.
Videographer's Note: Shot 4.2.1 (From #67679)

3.2.2. Talent carefully pipetting the prepared sample into a long single well.
Videographer's Note: Shot 4.2.1 (From #67679)

3.3. Perform electrophoresis at a constant current of 50 milliamperes per gel **[1]** until the dye front reaches the bottom of the gel **[2]**. After electrophoresis, equilibrate the gel in elution

buffer for 10 minutes **[3]**.

3.3.1. Talent setting the electrophoresis parameters.
Videographer's Note: Shot 4.2.2 and added shot 4.2.2-2(From #67679)

3.3.2. Shot of the dye front reaching the bottom line on the gel.

3.3.3. Talent transferring gel to a tray with elution buffer and gently shaking it on a rocker.
Videographer's Note: Shot 4.3.1 (From #67679)

3.4. Transfer the gel to a whole gel eluter apparatus as per the instrument manufacturer's instructions **[1]**. Run the eluter at a constant current of 250 milliamperes for 1 hour **[2]** to elute the proteins from the gel **[3].**

3.4.1. Talent positioning the equilibrated gel into the whole gel eluter system following a printed manual.
Videographer's Note: Shot 4.3.1 and 4.3.2 (From #67679)

3.4.2. Shot of the current being set on the eluter.
Videographer's Note: Shot 4.3.2 (From #67679)

3.4.3. Shot of a fraction being eluted.
Videographer's Note: Shot 4.3.3(From #67679)

3.5. Then use a vacuum pump to collect approximately 30 protein fractions, each measuring 3 milliliters, from the gel **[1]**.

3.5.1. Talent collecting eluate into tubes labeled 1 to 30.
Videographer's Note: Shot 4.4.1 (From #67679)

3.6. Quantify the protein concentration in the eluted fractions using the bicinchoninic acid protein assay **[1]**.

3.6.1. Talent performing BCA assay with standards and samples, measuring absorbance using a microplate reader.
Videographer's Note: Shot 2.7.1 (From #67679)

3.7. Subject 10 micrograms of the eluted protein fractions to SDS-PAGE analysis and visualize the bands by Coomassie Brilliant Blue staining **[1]**.

3.7.1. Shot of separated fractions on a PAGE gel, post staining.

Results

4. **Results**

4.1. Distinct protein banding patterns were observed in the 1st supernatant, cell wall, cell membrane, and cytosol fractions of *Mycobacterium tuberculosis*, confirming successful subcellular fractionation **[1]**.

4.1.1. LAB MEDIA: Figure 1. *Video editor: Please sequentially highlight the lanes labeled "1'st SUP", "CW", "CM", "Cytosol"*

4.2. The isoelectric focusing of cell wall proteins showed the highest protein concentration in fractions with pH below 2.5 **[1]**. SDS-PAGE of the cell wall IEF fractions revealed distinct protein bands predominantly in fractions 1 to 8 **[2]**.

4.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the first three data points at the leftmost end of the curve*

4.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the lanes 1 through 8*

4.3. Cell membrane protein separation by IEF revealed major protein concentration peaks between pH 4 and pH 10, with the highest concentration occurring around pH 10 **[1]**.

4.3.1. LAB MEDIA: Figure 4. *Video editor: Emphasize the data points between 4 to 10*

4.4. SDS-PAGE of the cell membrane IEF fractions showed diverse protein profiles, with clearer bands in fractions 1 to 9 **[1]**.

4.4.1. LAB MEDIA: Figure 5. *Video editor: Highlight lanes 1 through 9*

4.5. Preparative SDS-PAGE separation of the IEF-resolved cell wall proteins produced fractions predominantly under 100 micrograms per milliliter **[1]**. SDS-PAGE of the eluted cell wall protein fractions displayed a wide range of protein molecular weights, with more concentrated bands in fractions 6 to 10 **[2]**.

4.5.1. LAB MEDIA: Figure 6. *Video editor: Highlight the tallest bar at "<50" on the x-axis.*

4.5.2. LAB MEDIA: Figure 7. *Video editor: Highlight lanes 6 to 10*

4.6. Preparative SDS-PAGE of cell membrane protein fractions revealed that over half of the fractions had protein concentrations between 50 and 199 micrograms per milliliter **[1]**. SDS-PAGE of the eluted cell membrane protein fractions revealed concentrated bands in lanes 1 to 8 and 13 to 16 **[2]**.

4.6.1. LAB MEDIA: Figure 8. *Video editor: Highlight the tallest bar labeled "50–99" on the x-axis.*

4.6.2. LAB MEDIA: Figure 9. *Video editor: Focus on lanes 1 to 8 and 13 to 16.*

Pronunciation Guide:

1. Mycobacterium
- **Pronunciation link:** https://www.howtopronounce.com/mycobacterium-tuberculosis
- **IPA (American):** */ˌmaɪkoʊbækˈtɪriəm/*
- **Phonetic Spelling:** my-KOH-bak-TEER-ee-əm

2. Tuberculosis
- **Pronunciation link:** https://accenthero.com/app/pronunciation-practice/english/american/tuberculosis
- **IPA (American):** */təˌbɝkjəˈloʊsɪs/*
- **Phonetic Spelling:** tuh-BER-kyuh-LOH-sis

3. Proteomic
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */ˌproʊtiˈoʊmɪk/*
- **Phonetic Spelling:** proh-tee-OH-mik

4. Isoelectric
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */ˌaɪsoʊɪˈlɛktrɪk/*
- **Phonetic Spelling:** eye-soh-ee-LEK-trik

5. Electrophoresis
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */ɪˌlɛktroʊfɔːˈriːsɪs/*
- **Phonetic Spelling:** ih-LEK-troh-fuh-REE-sis

6. Hydrophobic
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */ˌhaɪdrəˈfoʊbɪk/*
- **Phonetic Spelling:** hy-druh-FOH-bik

7. Denaturation
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */diˌneɪtʃəˈreɪʃən/*
- **Phonetic Spelling:** dee-NAY-chuh-RAY-shuhn

8. Glycerol
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */ˈglɪsəˌrɒl/* or *US: /ˈglɪsəˌrɔl/*
- **Phonetic Spelling:** GLIS-uh-rol

9. Digitonin
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */ˌdɪdʒɪˈtoʊnɪn/*
- **Phonetic Spelling:** dij-ih-TOH-nin

10. Coomassie
- **Pronunciation link:** No confirmed link found
- **IPA (American):** */kuːˈmæzi/*
- **Phonetic Spelling:** koo-MAH-zee