Optimization of sample preparation in proteomic analyses using suspension trap digestion

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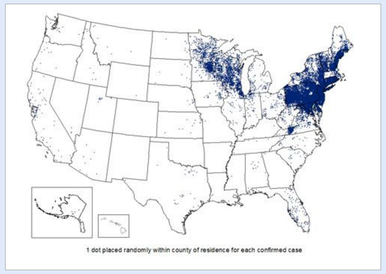
Context

Lyme Disease Overview

Lyme disease is a multi-organ illness caused by the spirochete *Borrelia burgdorferi,* which is transmitted to humans via tick bites. The disease was traditionally prevalent in the Northeast region, but it is now found in all fifty states and has become the most common vector-borne illness. Cases have risen to over 300,000 each year (Center for Infectious Disease Control and Prevention). Thus, to address the rapid spread of Lyme disease and the unknown causes of its chronic form (Post-Treatment Lyme Disease Syndrome), researchers at ISB are studying the condition through many approaches including bottom-up proteomic analysis.



Reported cases of Lyme disease in 2016
https://www.cdc.gov/lyme/stats/maps.html

1 dot placed randomly within county of residence for each confirmed case

Objective

Sample Preparation:

Out with the Old and In with the New?

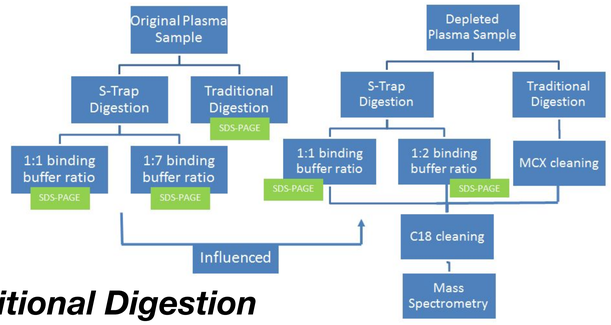
Plasma samples from Lyme disease patients are currently prepared for mass spectrometry using a traditional in-solution trypsin digestion. However, the S-Trap (suspension trap) digestion procedure from the biotechnology company, ProtiFi, may have several advantages over the current method.

Potential S-Trap Advantages:

- **Greater peptide yield**
  - No sample concentration step necessary
- **Higher efficiency**
  - No overnight digestion
  - Use of strong reagents eliminates post-elution cleanup steps
- **Better reproducibility**
- **Lower cost**

To test these potential advantages, the S-Trap was adjusted for use with plasma samples similar to those of Lyme patients. To determine the more favorable method, S-Trap digestion was compared to traditional digestion in terms of peptide recovery.

Experimental Design



Traditional Digestion

Unlike the S-Trap, in-solution digestion does not use any filter apparatus. Instead, it consists of adding various reagents in a combined solution and incubating.

In-Solution Procedure

1. Combine the sample with NH_4HCO_3.
2. Add TFE, vortex, and incubate at 55ºC for 30 minutes.
3. Add TCEP and incubate at 55ºC for 30 minutes.
4. Add iodoacetamide and incubate at 37ºC for 30 minutes.
5. Add NH_4HCO_3 and trypsin and incubate at 37ºC overnight with gentle shaking.

The required overnight incubation takes a significant amount of time, ultimately hindering the sample preparation process. In addition, traditional digestion for Lyme plasma samples requires a prior concentration step that tends to lower the final peptide yield.

Revised S-Trap Procedure

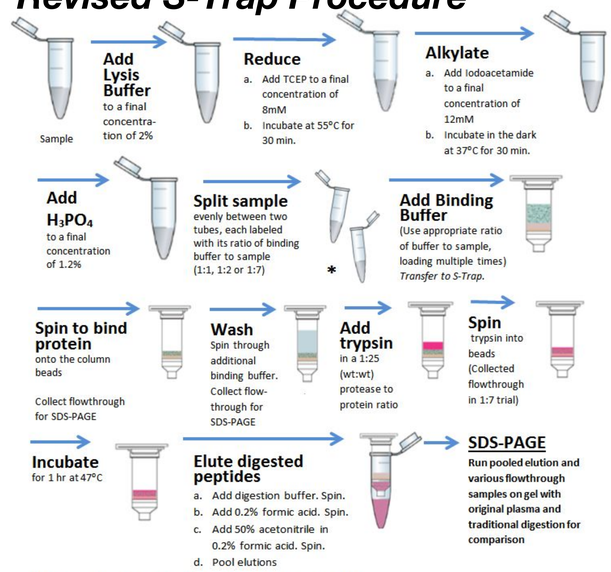


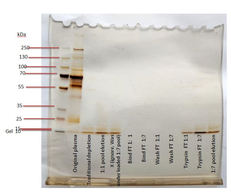
Image adapted from ProtiFi, LLC.

Data and Results

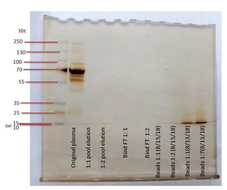
Qualitative Analysis

SDS-PAGE gels and silver staining were used to evaluate the loss of proteins:

- In flowthrough from the binding and wash steps
- From incomplete digestion/elution (tested from proteins left on S-Trap column beads)



Protein bands from a gel run on an original plasma sample.



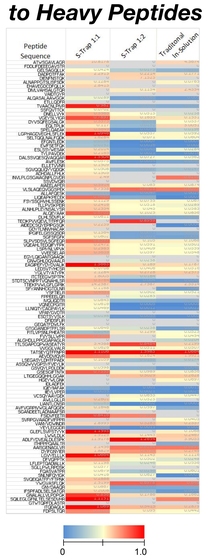
Protein bands from a gel run on a depleted plasma sample.

SDS-PAGE Gel Results

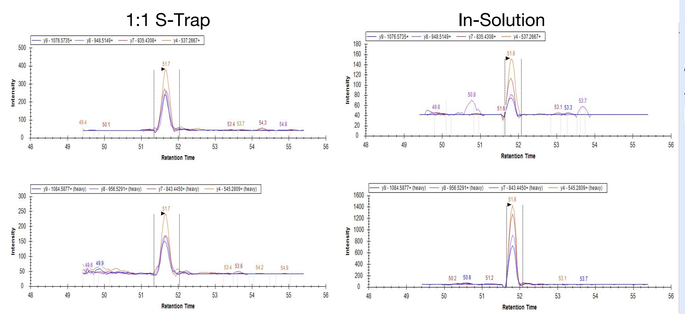
- Both binding buffer ratios, 1:1 and 1:7, yielded the same amount of proteins. Therefore, the volume of the binding buffer can be decreased to conserve the reagents and for greater efficiency (fewer loads).
- Loss of proteins is minimal during the binding and wash steps of the S-Trap protocol.

Quantitative Analysis

Area Ratios of Endogenous to Heavy Peptides



Example Mass Spectrum
APOA4 protein, SELTQQLNALFQDK peptide



Mass spectrometry was used to introduce a highly sensitive and numerical measurement of protein recovery. The heat map is based on data provided by mass spectrometry, demonstrating the area ratios of endogenous to heavy (sample to standard) peptides. From the data, the S-Trap 1:1 method demonstrates a higher protein yield than the S-Trap 1:2 and the traditional digestion techniques.

Design Reasoning

Obstacles of Original S-Trap Protocol

The following concerns were involved with ProtiFi's given protocol for S-Trap Digestion:

1. **Side effects due to overuse of strong chemicals for protein breakdown**
   Unlike tissue samples for which the ProtiFi protocol is written, plasma samples lack cellular materials that require breakdown. This permits the decrease of strong reagents used in steps preceding the addition of protease.
2. **Low protein concentration within the sample**
   ProtiFi's protocol requires a 1:7 sample to binding buffer ratio. However, since depleted plasma samples already have high volumes due to low protein concentration, a 1:7 ratio requires the experimenter to load samples multiple times. Lower binding buffer ratios were tested to determine one that prevented this inefficiency while maintaining the binding power of the S-Trap.
3. **Incomplete protein digestion and/or peptide loss**
   To thoroughly evaluate the accuracy of the S-Trap column, each step was tested for loss of proteins. Spin flowthrough was not disposed. Instead, both flowthrough from various steps and the final elution were collected and tested via SDS-PAGE gel. Potentially undigested proteins remaining on the S-Trap beads were also run on gels.

Conclusions

- Based on the data collected, the S-Trap provides a sufficient alternative to in-solution digestion: retained peptides were at similar or higher levels than the traditional method. In particular, the S-Trap method with a binding buffer to sample ratio of 1:1 had the best peptide retention in this trial.
- The S-Trap protocol can be applied to larger volume depleted plasma samples without interfering with efficiency and accuracy.
- Further experimentation is necessary to confirm these results.

Future Directions:

Additional control factors and trials are necessary to avoid potential variation.

- Additional trials should re-evaluate:
  - Effect of higher binding buffer ratios on protein retention
  - Mass spectrometer retention time
  - Overall comparison of in-solution and S-Trap
- New control factors:
  - Single batch for post-digestion C18 cleanup

References

1. Center for Disease Control and Prevention. (2018, January 19). Lyme Disease. Retrieved August 19, 2018, from https://www.cdc.gov/lyme/index.html
2. Wilson, J. (2016). S-Trap™. Retrieved August 19, 2018, from https://www.protifi.com/s-trap/

Acknowledgments

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