Adaptation of the TruCulture® System for Flow Cytometry Analysis of Leukocyte Activation Markers

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Introduction

Evaluation of the immune system is essential in medical research and clinical patient care. Enhancement of immune responses to tumor cells is currently the most promising and active area in cancer drug development. While it is advantageous to assay immune cells as soon as possible after specimen collection, complex laboratory procedures are difficult to implement in multi-site clinical studies. The TruCulture® tube (Rules-Based Medicine, Inc., Austin TX) was developed to be a simple, complete and closed blood collection system. The system enables standard and simple activation and processing of blood samples to determine cytokine release into plasma. The purpose of this study was to determine if the TruCulture® system could be adapted for subsequent flow cytometry assay of leukocyte cell surface antigens. We also investigated antigen stability of activated samples and evaluated techniques for providing sufficient stabilization for later testing. Given that TruCulture® tubes require antigen stability of activated samples and evaluated techniques for providing sufficient stabilization for later testing.

Methods

Blood Collection and Activation

1. Bring TruCulture tube to room temperature
2. Pull back syringe to engage vacuum in tube
3. Using butterfly needle with adapter to draw blood sample drawn directly into TruCulture tube
4. Invert tube to mix
5. Snap off plastic syringe
6. Place tube upright in 37°C heat block

Blood Collection and Activation

Figure 1. Flow Cytometry

Figure 2. Phenotyping Results and Troubleshooting

Figure 3. Leukocyte Activation Results

Figure 4. Stability of Monocyte and Neutrophil Activation

Figure 5. Stability of Lymphocyte Activation

Summary and Conclusions

Healthy volunteer blood samples cultured in TruCulture® tubes showed strong upregulation of HLA-DR on monocytes (75-90% in 3 hr) and CD11b (20-40% in 24 hr) and CD66b (10-30% in 24 hr) in T lymphocytes.

In general, activation signals from these samples may need to be stabilized if not obtained immediately, e.g. monocyte HLA-DR signal in unstimulated “Null” samples increased over time in unfixed samples and, without fixation, neutrophil CD11b increased during the first 48 hours after culture.

Our findings demonstrate that both plasma cytokine and flow cytometry analyses can be obtained from a single TruCulture® sample with minimal added manipulation. This approach should be expanded with new markers and cell stimulants to provide in depth analysis of immune responses, particularly in the clinical trial setting.

Antibodies

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<tr>
<th>Antibody Name</th>
<th>FLUOROCHROME</th>
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<tbody>
<tr>
<td>Monocyte and Neutrophil Panel (CD11b, CD14, CD16, CD45, CD62L, HLA-DR)</td>
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<td>FL1 FL2 FL3 FL4 FL5 FL6 FL7 FL8</td>
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<td>BV421 V500 FITC PE PerCP PE-Cy7 APC APC-H7</td>
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Methods

Materials

- Blood samples
- TruCulture® tubes
- Flow cytometry equipment
- Antibodies

Protocols

1. Blood collection
2. Activation in TruCulture® tubes
3. Flow cytometry
4. Data analysis

Results

- Leukocyte activation
- Monocyte and Neutrophil activation

Discussion

- Stability of activation signals
- Comparison with traditional methods

Conclusion

- Adaptation of TruCulture® for flow cytometry
- Advantages of closed system

References