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Investigations into Aldefluor as a Novel Method for Identifying Leukemia in Soft-Shell Clams

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Investigations into ALDEFLUOR® as a Novel Method for Identifying Leukemia in Soft-Shell Clams

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Introduction

The clam species *Mya arenaria* is a common model organism in leukemia research. The current method for classifying the degree of cancer progression is by examining cell morphology with light microscopy. This approach is highly qualitative, which makes differentiation of pre-leukemic and semi-leukemic individuals difficult. One quantitative approach that may differentiate individuals is based on levels of aldehyde dehydrogenase (ALDH) expression. The enzyme assay ALDEFLUOR® can actively measure ALDH expression in viable cells, but the effectiveness of certain protocol conditions is dependent upon the cell type.

Aims of the Study:

- To find the ideal conditions for the ALDEFLUOR® enzyme assay with field samples.
- To determine the viability of the assay as an alternative method to the standard visualization procedure.

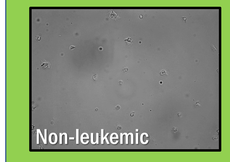
Research Questions:

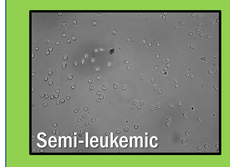
Under what conditions does the Aldefluor assay provide the best results for cell samples taken from soft-shell clams?

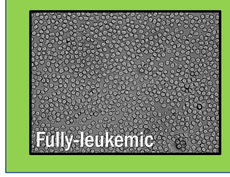
Of the two methods, which one is best for identifying cancerous clam cells?

Results and Data Analysis

Hemolymph Imaging: As leukemia develops, hemolymph biopsies reveal subtle changes in cell morphology to rounder, smoother cells, as well as drastic increases in cell concentration.



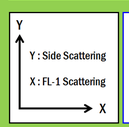


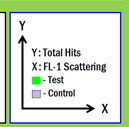


Aldefluor Scatterplots: The top row has control groups, which demonstrate the baseline fluorescence of a cell with DEAB-inhibited ALDH. The second row has experimental groups, which demonstrate the fluorescence of cells with uninhibited ALDH. Each column has a specific set of protocol conditions, but these are just a small subset of the total collected data.

Legend:

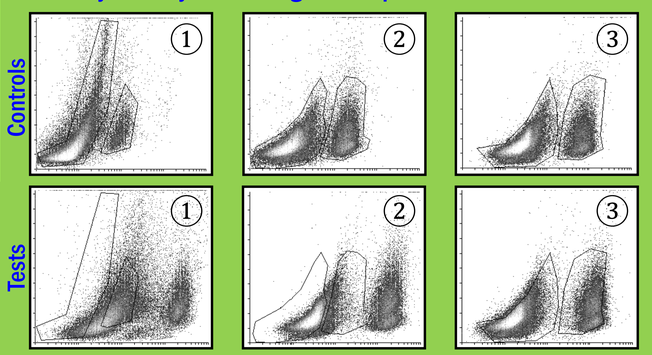
1. **- Non-leukemic Clam, 1 x 10^5 cells/mL, 45 min.**
2. **- Leukemic Clam, 2 x 10^6 cells/mL, 30 min.**
3. **- Leukemic Clam, 2 x 10^6 cells/mL, 45 min.**



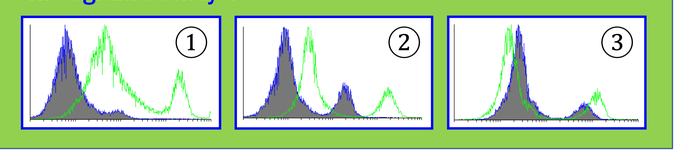


Histogram Overlays: The histograms demonstrate the number of cells, regardless of size, which are scattering the laser at a specific wavelength. This allows for a direct comparison of the overall fluorescence between control and experimental samples. Under ideal conditions, the size of the overlap between control and experimental samples is minimized for both cell populations, indicating a large difference in fluorescence between samples.

Flow Cytometry Scattering Technique:



Histogram Overlays:

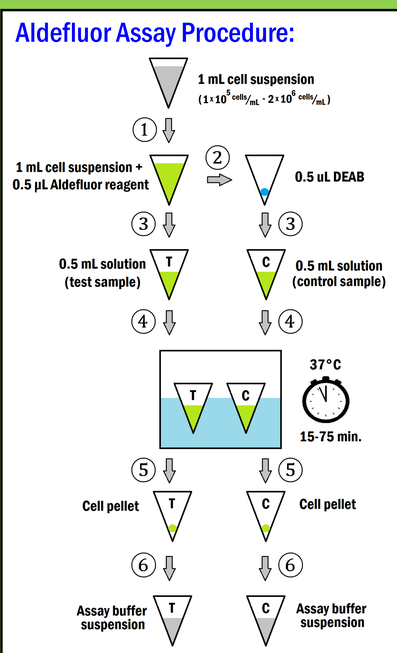


Materials and Methods

Microscopy: In the traditional method, hemolymph samples are examined directly using a compound light microscope.

1. Add 0.5 uL Aldefluor reagent
2. Quickly transfer 0.5 ml to control
3. Mix & label
4. Incubate samples
5. Centrifuge & remove liquid supernatant
6. Resuspend in assay buffer

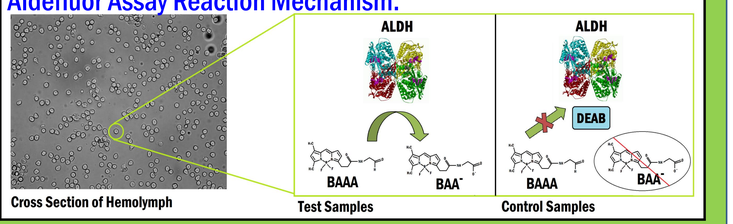
Aldefluor Assay Procedure:



The Aldefluor Assay: For the novel method, BAAA is added to a concentrated cell suspension. Half of this is transferred to the DEAB control tube. Both samples are incubated at 37°C for 15-75 min. and then centrifuged for 5 min. at 10,000g before resuspension in assay buffer.

Flow Cytometry: Each solution sample is run through a flow cytometer. This machine uses laser scattering to detect the size, granularity, and fluorescence of individual cells. These properties are directly proportional to the amount of light that is scattered.

Aldefluor Assay Reaction Mechanism:



Reaction Mechanism: ALDH converts the Aldefluor reagent (BAAA) to its fluorescent form (BAA^-). In the control-treated samples, DEAB prevents BAAA from binding to ALDH. Thus, no BAA^- is generated, and the amount of fluorescence is reduced.

Conclusions

The Aldefluor assay alone cannot be used to identify the level of leukemic progression with an individual clam.

There are two distinct cell populations in every hemolymph sample, each with a different capacity for ALDH expression. These cell populations can only be identified through the Aldefluor assay, and not through visualization.

The protocol conditions needed for ideal results will be different based on the level of leukemic progression within the clam.

- **Sample 1** describes the best results for a **non-leukemic** clam.
- **Sample 2** describes the best results for a **leukemic** clam.

Sample 3 shows the results of non-ideal assay conditions, while also demonstrating an interesting anomaly for further investigation.

References & Acknowledgements

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