Automated flow cytometry for medical diagnostics

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Abstract

Medical diagnostic flow cytometry is currently limited to high complexity laboratory settings because preanalytical steps are time consuming, exacting and require highly trained and capable technologists to minimize variability. Individual instrument, and instrument-to-instrument variability limit broad acceptance of flow cytometry testing by users and regulatory bodies. Finally, interpretation of flow cytometry results requires highly trained flow cytometrists who are typically available only during normal work hours.

Sepsis confirmation using an assay to detect activated neutrophils based on up regulation of CD64 would benefit from 24/7 availability of flow cytometry capability. Lack of this capability limits the use of such an assay as an aid to sepsis confirmation since each hour of delayed treatment increases mortality by 7%. The disposable cartridge-based platform described in this paper resolves the limitations of conventional flow cytometry to provide 24/7 availability in a moderate complexity, and ultimately CLIA waved setting. Once a sample is introduced into the closed cartridge all preanalytical and analytical processing is performed in the cartridge without further user intervention. Post analytical processing of the flow cytometric data collected by the companion reader is automatically analyzed to produce a final assay report for the medical practitioner.

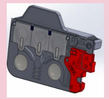
Details of the novel microfluidic-based cartridge and its automatic operation controlled by the companion reader are presented. Results comparing the analytical performance of a CD64 assay using this instrument to that of conventional flow cytometry are presented showing an R^2 exceeding 0.95. Also shown is the typical minimal user interaction required to run the instrument and obtain results. Thus, key moderate complexity requirements are demonstrated.

Objective: Answer, "Why send out flow cytometry tests and wait 12-48 hours for results?"

- Hospitals without high complexity lab certification must send out their specimens to qualified labs
  - Limits flow cytometry to non-stat applications
  - Increases laboratory costs
- Of 6000 hospital labs only 2000 are high complexity certified
- Even in these 2000 labs flow cytometry may only be available 5 days per week from 9 AM to 5 PM
- Moderate complexity flow cytometry with 24/7 availability is an unmet need

What is our answer?

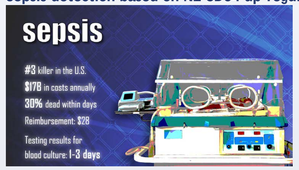
Automated Sample Preparation + **Automated Reading & Data Analysis**



Relevance to clinical laboratory practice

What is our first application?

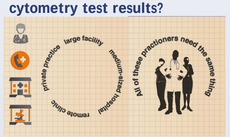
Sepsis detection based on NE CD64 up-regulation



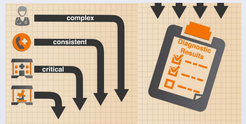
Davis, Bruce H., et al. "Neutrophil CD64 is an improved indicator of infection or sepsis in emergency department patients." *Archives of pathology & laboratory medicine* 130.5 (2006): 654-661.

Kingma, Paul S. "Laboratory and Clinical Practice for Monitoring Sepsis with Neutrophil CD64 Index."

Who needs fast access to flow cytometry test results?



What results are needed?

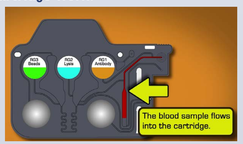


How is this accomplished?

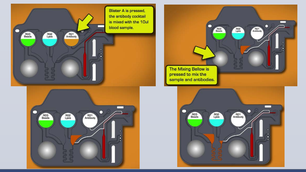
Put a drop of blood into the cartridge | Insert the cartridge into the reader | Wait 10 minutes and read the CD64 Index

Method

How does the cartridge work?

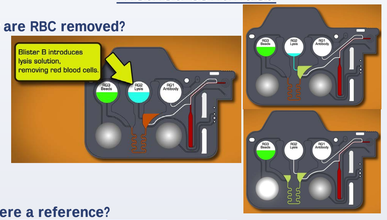


How is the antibody cocktail introduced?

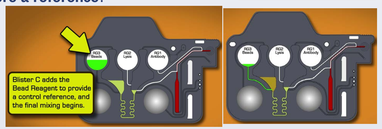


Method continued

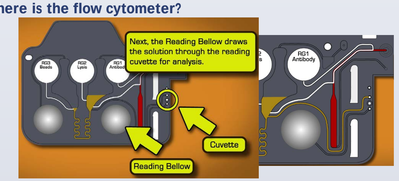
How are RBC removed?



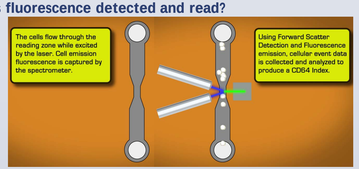
Is there a reference?



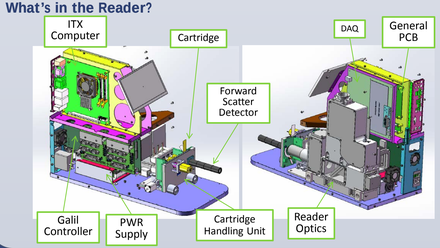
Where is the flow cytometer?



How is fluorescence detected and read?



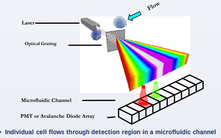
What's in the Reader?



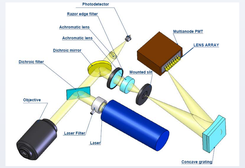
Method continued

What is the fluorescence detection technology?

Micro Flow Spectrometer



- Individual cell flows through detection region in a microfluidic channel
- Cells labeled with antibodies conjugated with multiple wavelength fluorescent tags
- Diode laser as illumination
- Optical grating disperses emission into its constituent wavelengths
- PMT or avalanche diode array detects fluorescence at 8 different spatial locations corresponding to 8 spectral colors

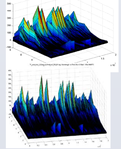


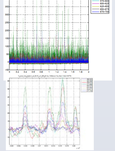
Epi fluorescence implementation

Results

What do typical fluorescent signatures look like?

Example: Reference bead detection signature





What is the fluor signature matrix A?

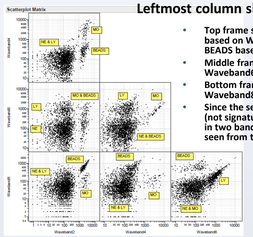
F488	Noise	PE488	SFR488	PEAF488
0.4738	0.3470	0.0108	0.0000	0.0133
0.8273	0.2082	0.0291	0.0000	0.0326
0.2784	0.8767	0.5925	0.0000	0.0734
0.1092	0.1425	0.7367	0.0000	0.0779
0.0384	0.1023	0.2906	0.0000	0.6976
0.0108	0.1023	0.1356	0.0000	0.6521
0.0106	0.1132	0.0450	0.5970	0.2448
0.0077	0.1169	0.0185	0.8023	0.1241

```
function [ x ] = FluorSolver( A, b )
%FluorSolver Solves for fluorophore composition of an observed 8-channel
%response
%   Detailed explanation:

%       This routine finds x, the solution of the equation Ax = b

%       A is a matrix with 8 rows whose columns are normalized fluor
%       responses, i.e. the sum or length of the 8-elements in each column is 1.

%       b is a column vector with 8-rows or a matrix of such column vectors
%       that is the observed response

%       x is a column vector with the number of rows equal to the number of
%       columns of A (number of possible fluors) if b is a column vector.  If
%       b is a matrix, x is a matrix with the number of rows equal to
%       the number of columns A and the number of columns equal to the
%       number of columns of b.  Each column of b is the weighting of the
%       fluors in the columns of A that best match the observed fluor
%       response.
%
%       Example:

   %A =                                          %b =
   %    1    10     5     0     0     0         %    1    11     5     0     0     0
   %    1     5    10     4     0     0         %    2    15    15     4     0     0
   %    0     0     3    10     5     0         %    2    20    13    10     5     0
   %    0     0    10     7     3     2

   %[ x ] = FluorSolve( A', b' )                %x =
                                                %    1.1191    1.0000    2.0000
   x = A\b;                                     %   -0.0416    1.0000    0.0000
                                                %    0.0301    0.0000    1.0000
   end                                          %   -0.0253   -0.0000   -0.0000
```

Results continued

What are the 8-channel results from a CD64 Assay?

Scatterplot matrix shows 4-color separation of NE, MO, LY and Beads

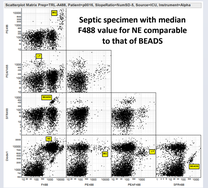


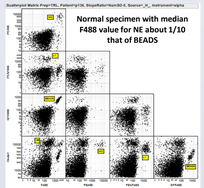
- Top frame shows separation of NE & LY from MO and BEADS based on Waveband2 (Alexa488) and separation of MO from BEADS based on Waveband4 (PE)
- Middle frame shows separation of LY from NE based on Waveband6 (A610)
- Bottom frame shows separation of beads from cells based on Waveband8 (Starfire Red)
- Since the separation is based on individual narrow bands (not signatures) 45 degree clusters show emission presence in two bands, which in each case is as expected as can be seen from the emission signatures in the table below

Waveband	F488	None	PE488	SFR488	PEAF488
1-500-524	0.269784	0.125	0.005805	0	0.006907
2-526-549	0.419081	0.125	0.015635	0	0.016996
3-550-574	0.138544	0.125	0.018743	0	0.018319
4-575-599	0.062158	0.125	0.396312	0	0.040674
5-600-624	0.021845	0.125	0.156359	0	0.364119
6-625-649	0.006157	0.125	0.072972	0	0.343878
7-650-674	0.006048	0.125	0.024219	0.426647	0.127784
8-675-700	0.004377	0.125	0.009968	0.573353	0.064779

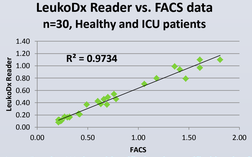
What are the results when fluor signatures are used?

Clear distinction of BEADS, MO, LY and NE for both Septic and Normal





How does the LeukoDx - CD64 Assay compare to that run on a flow cytometer?



Assay Results: We have identified and quantified levels of leukocyte neutrophil CD64 with a moderate complexity flow cytometer

What is our Conclusion?

Automated Sample Preparation + **Automated Reading & Data Analysis**

provides automated moderate complexity flow cytometry