



BVAC/ABCA

Annual Symposium

26 October 2007

Mons, BE

Dirk Van Bockstaele, Ph.D.
Director Flow Cytometry Laboratory,
European Headquarters

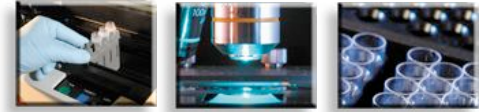


Multiparameter
strategies
in
rare event
analysis

ESOTERIX^U_Z
Clinical Trials Services

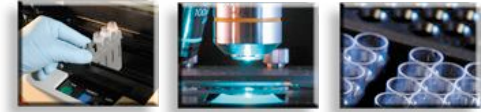
A LabCorp Company

Rare event FCM

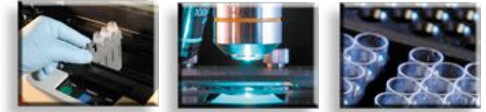


- \neq regular FCM
- Impossible to perform on the same machine in between regular FCM acquisitions
- Needs dedicated machines
- Those that disagree ...
- ... are badly applying flow cytometry
- ... are not aware of the typical “rare event” detection problems

Worth while reading



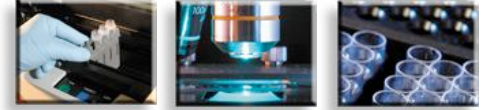
- Principles of rare event analysis by flow cytometry: Detection of injected dendritic cells in draining lymphatic tissue. AD Donnenberg and EM Meyer, *Clinical Immunology Newsletter* 19: 124-128, 1999.
- Detection of circulating endothelial cells and endothelial progenitor cells by flow cytometry. SS Kahn, MA Solomon, JP McCoy, *Cytometry* 64B: 1-8, 2005.



What is a rare event?

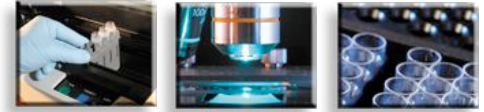
- Practical definition: all events of interest that are as frequent or less frequent as spurious events
- What is a spurious events?
 - Practical definition: all unfrequent artefactual flow events that have nothing to do with unlabelled or specifically (fluorescent-)labelled single viable cells
- What frequencies are we talking about?
 - From $< 0.1\%$ to as low as what is practically feasible
- What is feasible?
 - Assuming normal WBC of 5,000 c/ μ l and 100 % recovery
 - 1%: 5,000 found within 100 μ l of blood
 - 0.1%: 500 found within 100 μ l of blood
 - 0.01%: 50 found within 100 μ l of blood
 - 0.001%: 5 found within 100 μ l of blood
 - data file of 5 million events @ 5000 c/sec, 17 minutes acquisition
- How about accuracy and precision??

Rare event analysis: S/N improvement



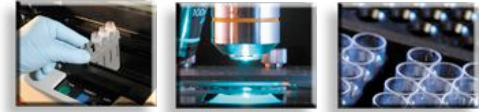
- Increase the frequency of the rare event of interest (signal):
 - Pre-enrichment techniques
- Decrease the frequencies of the spurious events (noise)
 - By preventing their occurrence
 - By excluding them during analysis

Spurious events

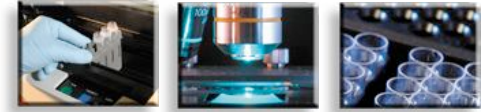


- Debris
 - Clean the cytometer
 - Use filtered solutions
- Non-specific labelled cell (non-specific binding of fluorochromes or ab)
 - Use blocking serum
 - Exclude dead cells
- Highly autofluorescent cell
 - Include at least one negative marking (or an unstained fl. parameter) for the rare event
 - Label all potentially interfering cells using a fl. « dump » channel.
- Non-nucleated cell
 - Exclude by a NA stain.
- Aggregates
 - Exclude by pulse shape analysis (area versus peak or width)
- Dead cells
 - Exclude by viability stain (membrane integrity stain)
- « space dust » (dixit Carlton Stewart)
 - Random (uncorrelated) noise and spikes from PMT's and electronics
 - Exclude by increasing the # of parameters that positively define the rare event
 - Define rare event with the brightest and tightest positive marking
- Transient flow disturbances (partial cloggings; clumps ...)
 - Excluded by time gating

Rare event analysis: double problem

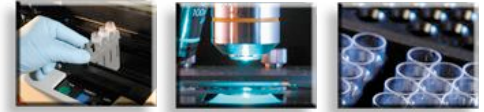


- Rare event problem obliges the need for **spurious event exclusion**
- Spurious event exclusion obliges the need of **multiparametric polychromatic flow**: 3 fluorescences is not enough!



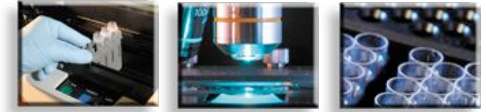
- And after all spurious events have been minimized or excluded ...

... characterize total noise and ...



- using an appropriate negative control
 - Isotype-matched irrelevant ab
 - (same conc., same F/P)
 - acquisition of the same amount of total events
 - Check what you find in the rare event gate
- This frequency of spurious events is the actual detection limit for your rare event
 - with a spurious event frequency of 0.02% (1 in 5000) , don't even try to detect the real rare event of interest at a freq. of 0.01% (1 in 10,000)!

... and report your precision ...



- Always report your precision

- Counting statistics // Poisson: Error $\sim \sqrt{\text{events}}$

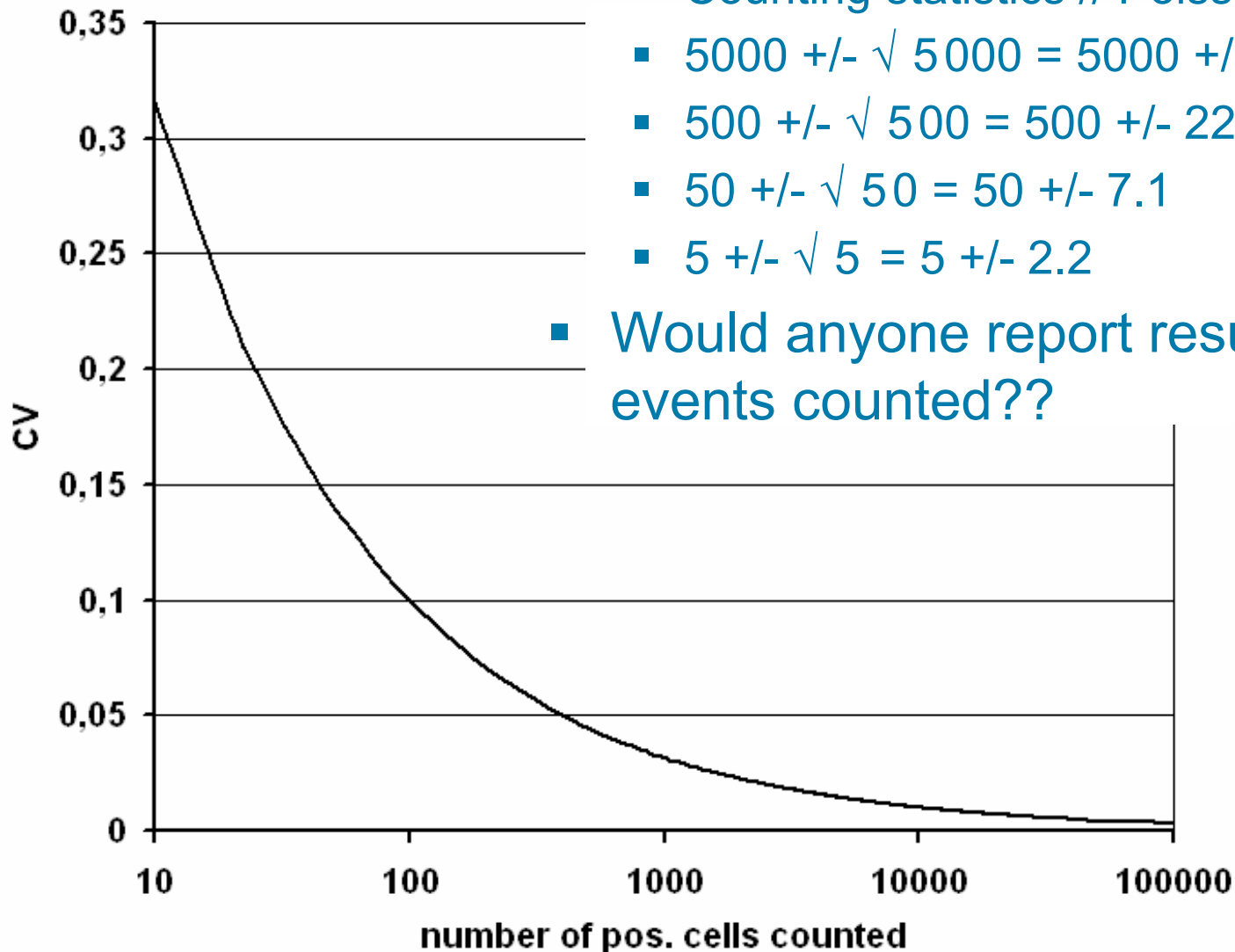
- $5000 \pm \sqrt{5000} = 5000 \pm 70.7$ (1%)

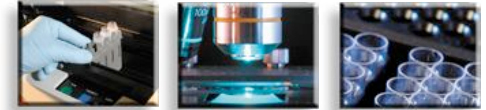
- $500 \pm \sqrt{500} = 500 \pm 22.4$ (4%)

- $50 \pm \sqrt{50} = 50 \pm 7.1$ (14%)

- $5 \pm \sqrt{5} = 5 \pm 2.2$ (45%)

- Would anyone report results based on 5 events counted??





Kijken Denken Doen (Look Think Do)

Lecture presented at the occasion of
the appointment as professor for

Medical Cell Biophysics

at the Faculty of Science and Technology
of the University of Twente
on the 27th of September 2007
by

Prof. Leon WMM Tenstappen, MD, PhD