

Clinical Flow Cytometry for the Perplexed

Part 5: Minimal (Measurable) Residual Disease Detection (MRD)

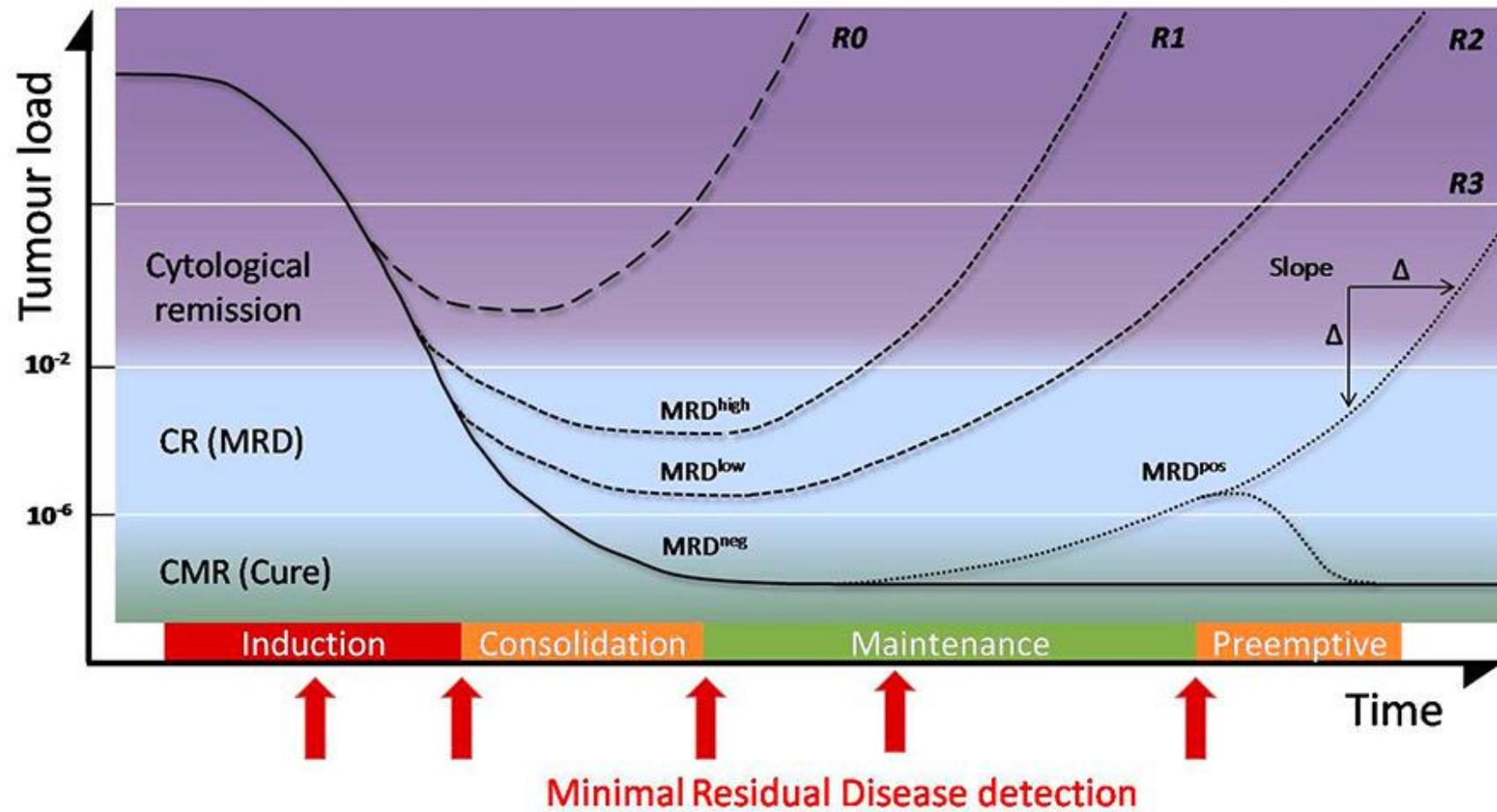
David P. Ng, MD

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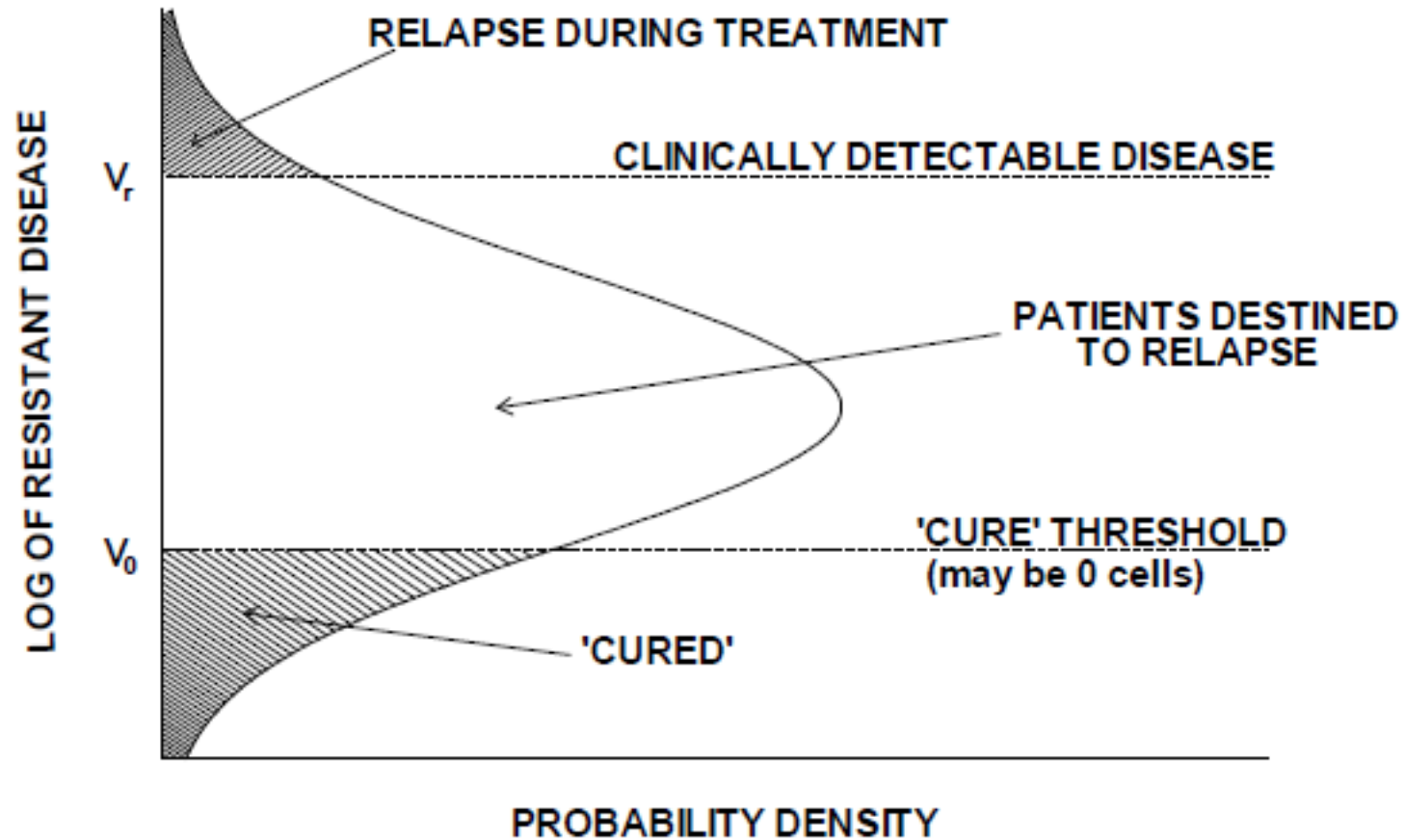
Goals

- Recognize the clinical significance of minimal residual disease
- Understand the statistical basis for detecting small populations

Why do you care?



Why do you care?



What is so hard about this?

- Numerator is the number of abnormal cells
- Denominator is the number of total cells

- You need to look at a lot of normal cells to find an abnormal cell



Rare Event Sampling

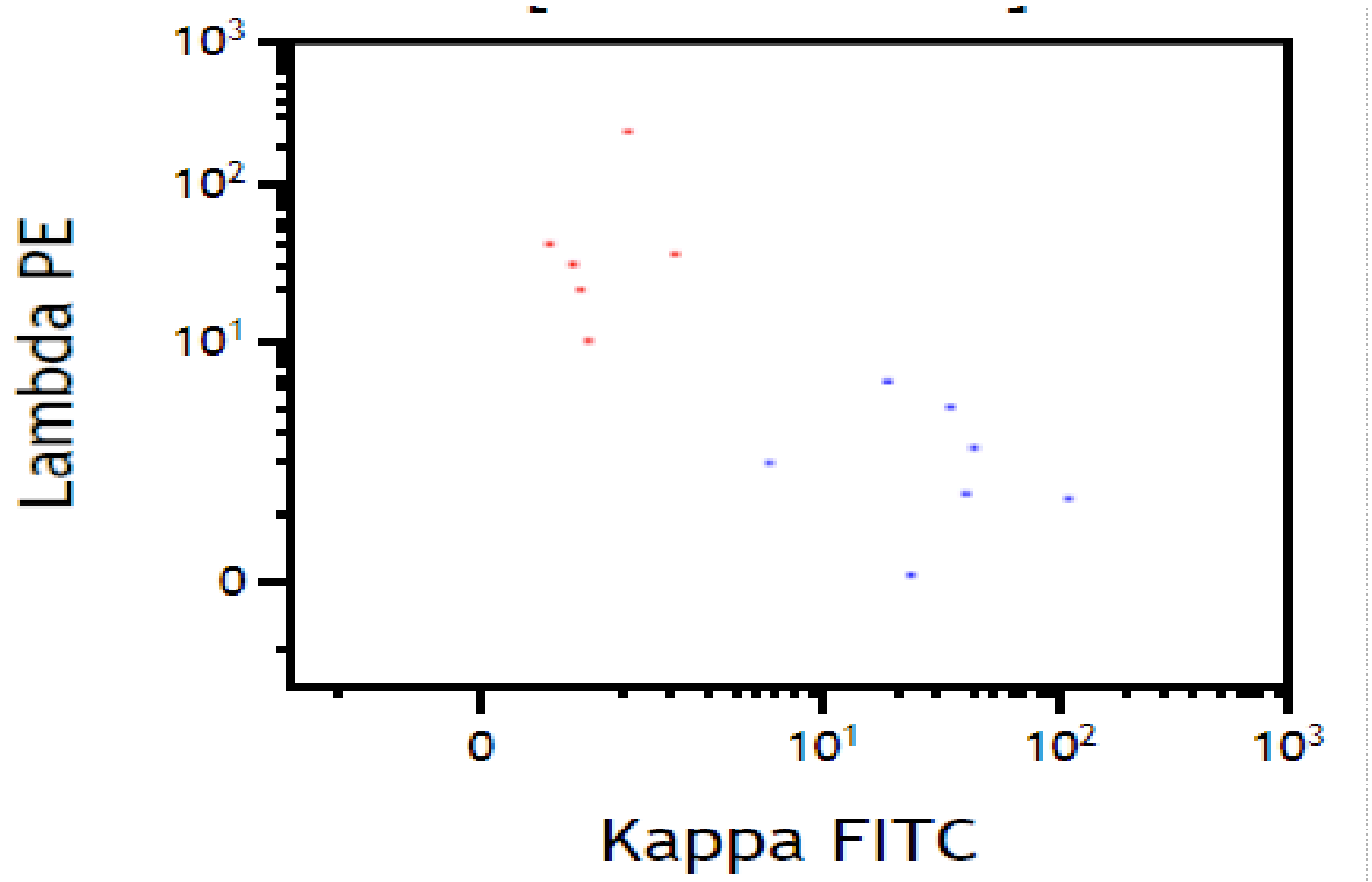
- Is this kappa lambda ratio significant?
 - » Do I have enough events to call ...
 - » Probably not...

- Is this population of cells significant to call minimal residual disease?
 - » Do I have enough events to call ...

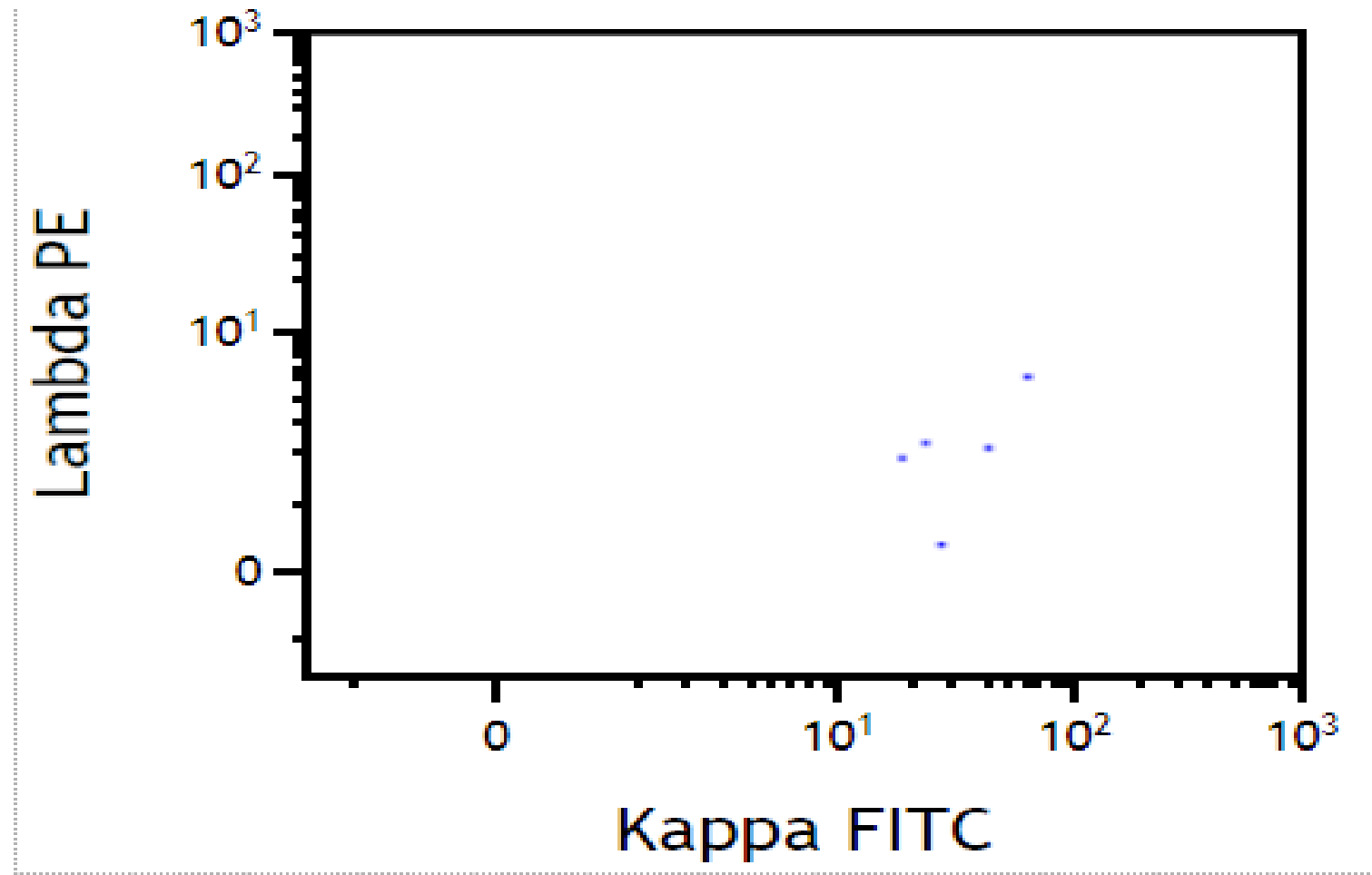
P_____y

- 11 letter word, Beginning in P, Ending in Y
- “I know it when I see it”
 - » Justice Potter Stewart on pornography
 - » Most physicians on probability

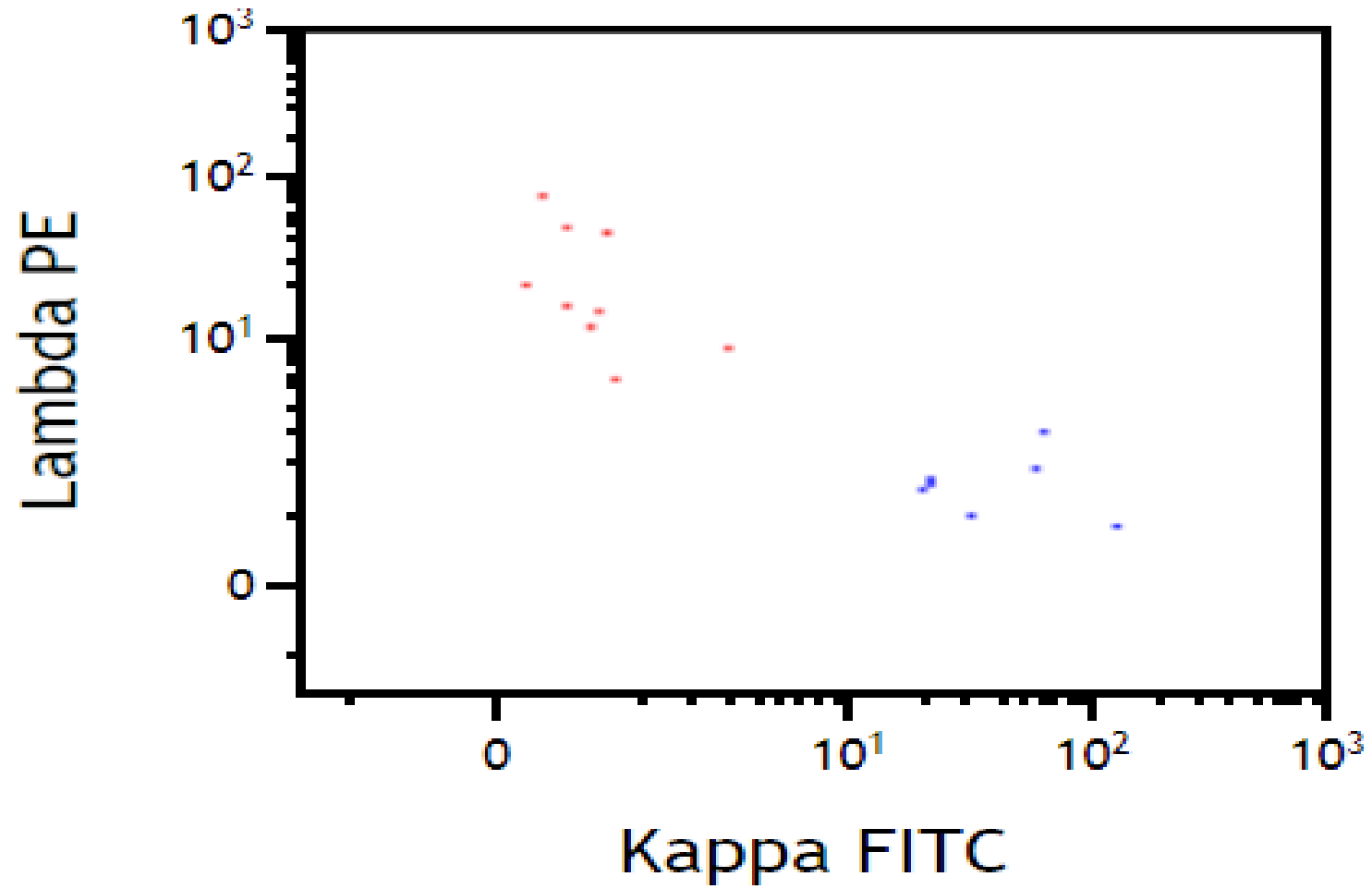
7:6



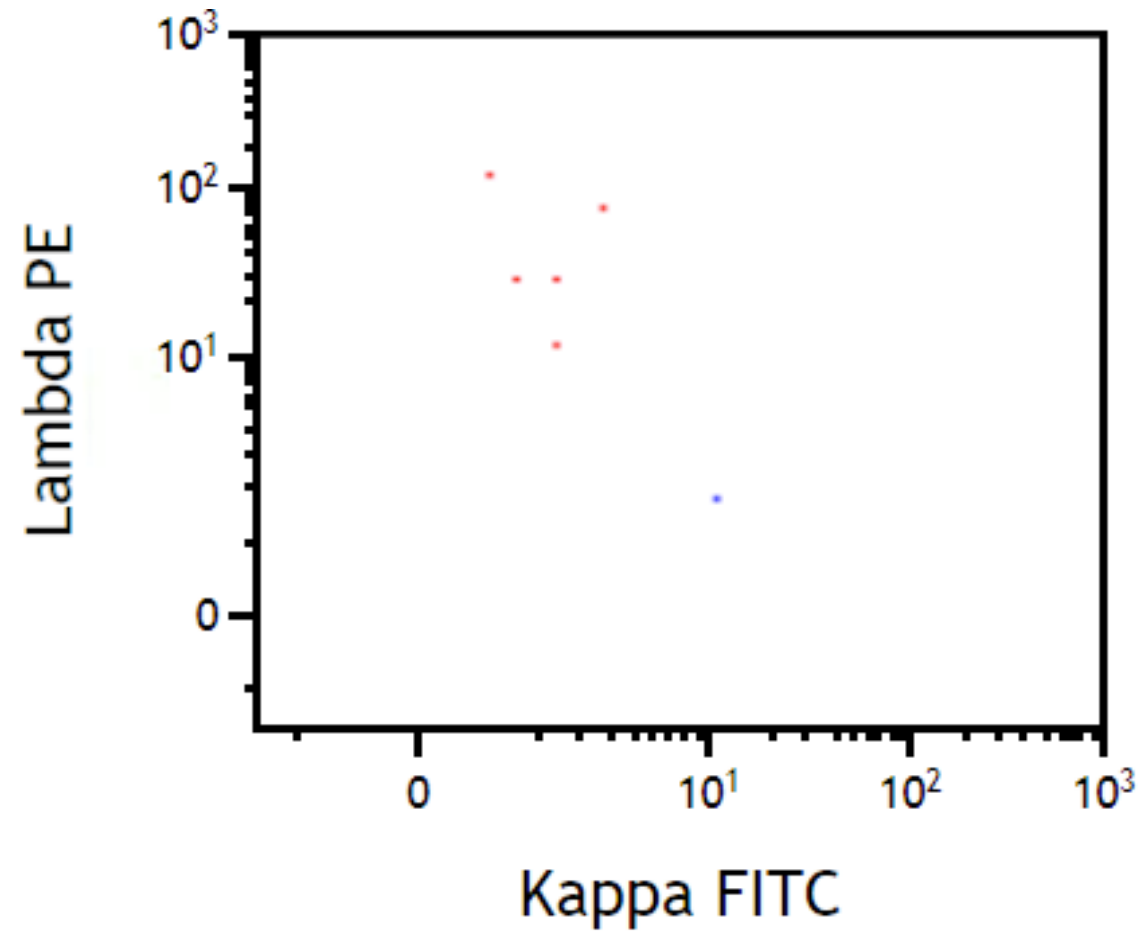
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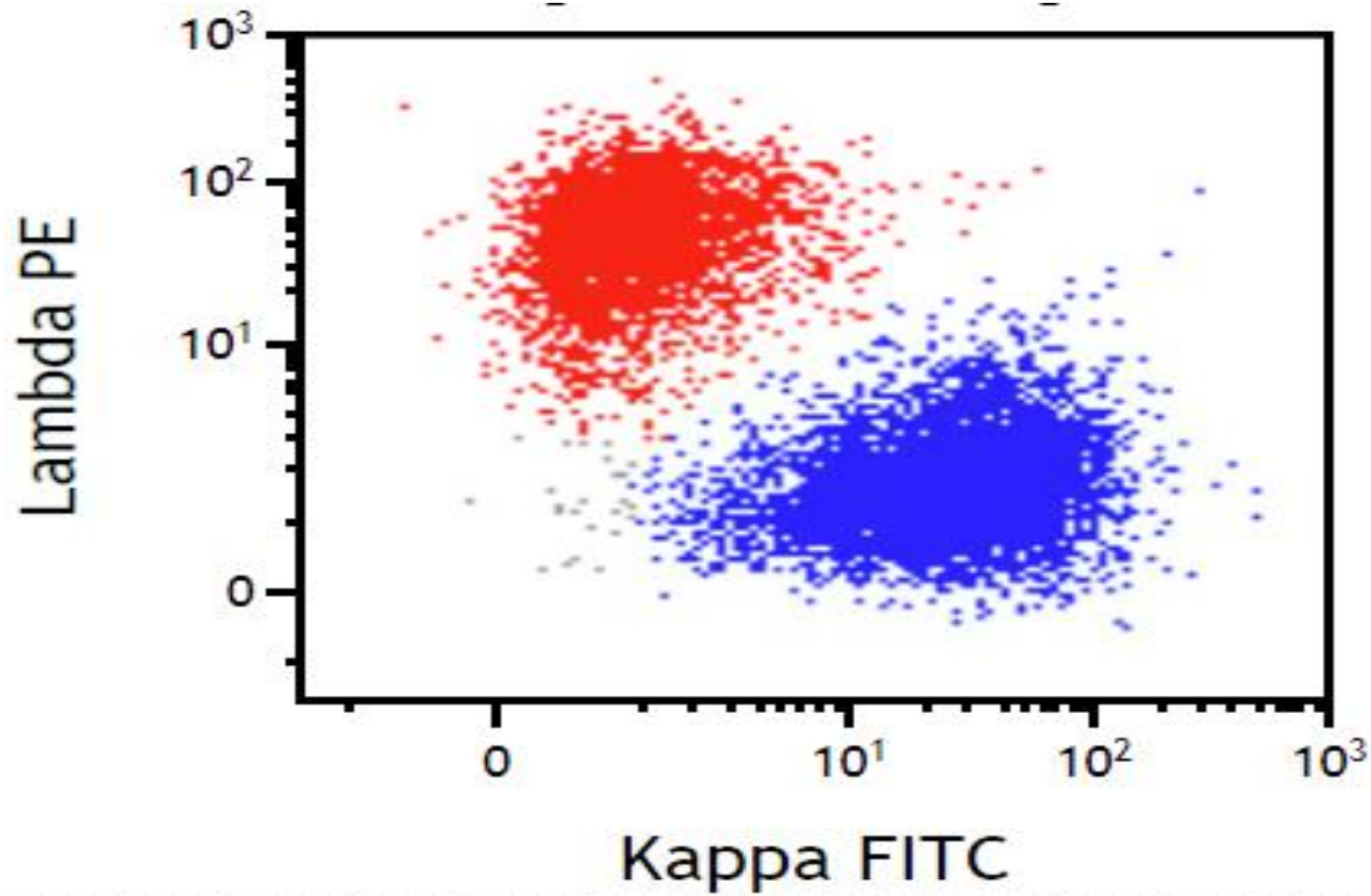
6:9



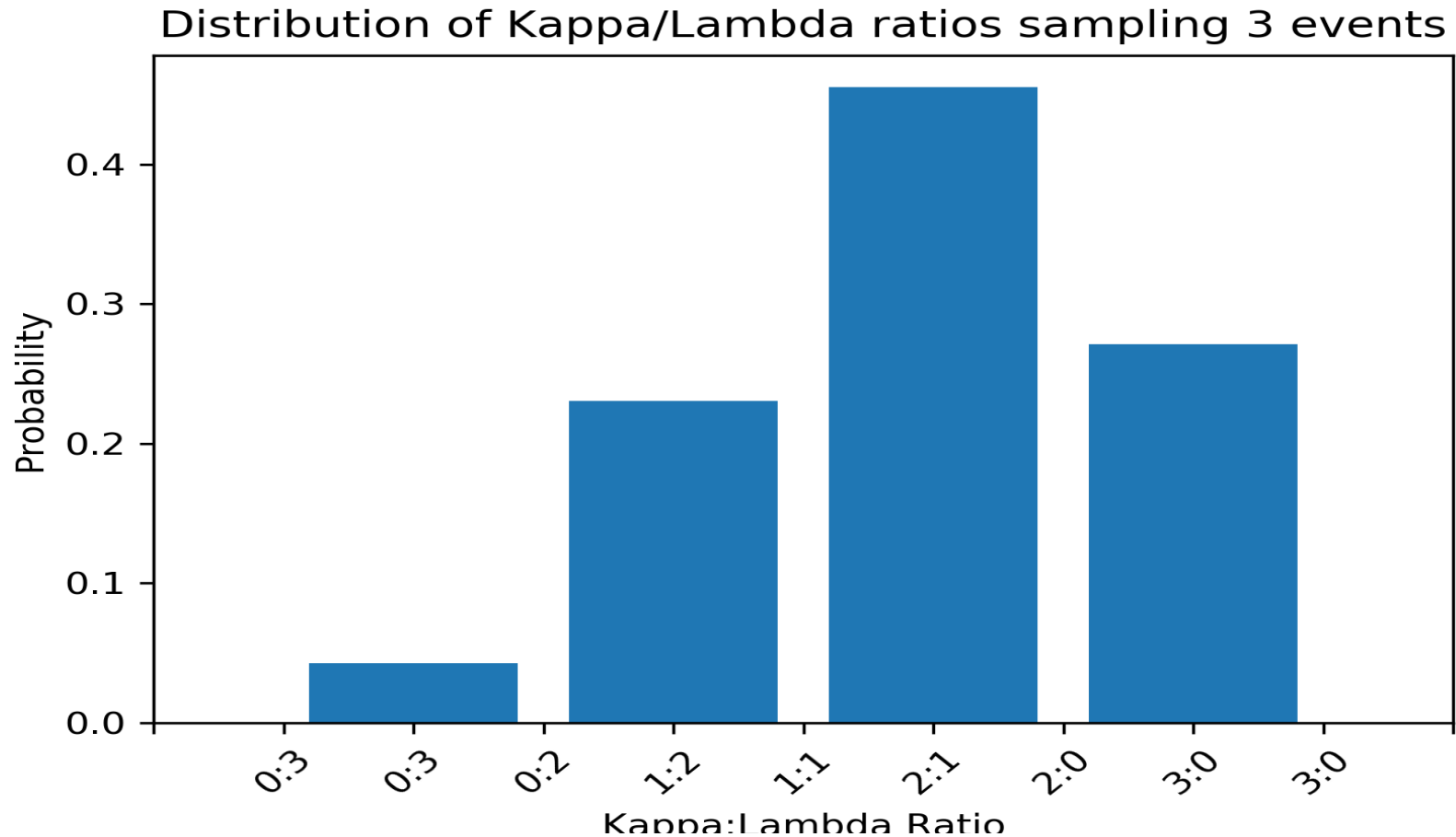
1:5



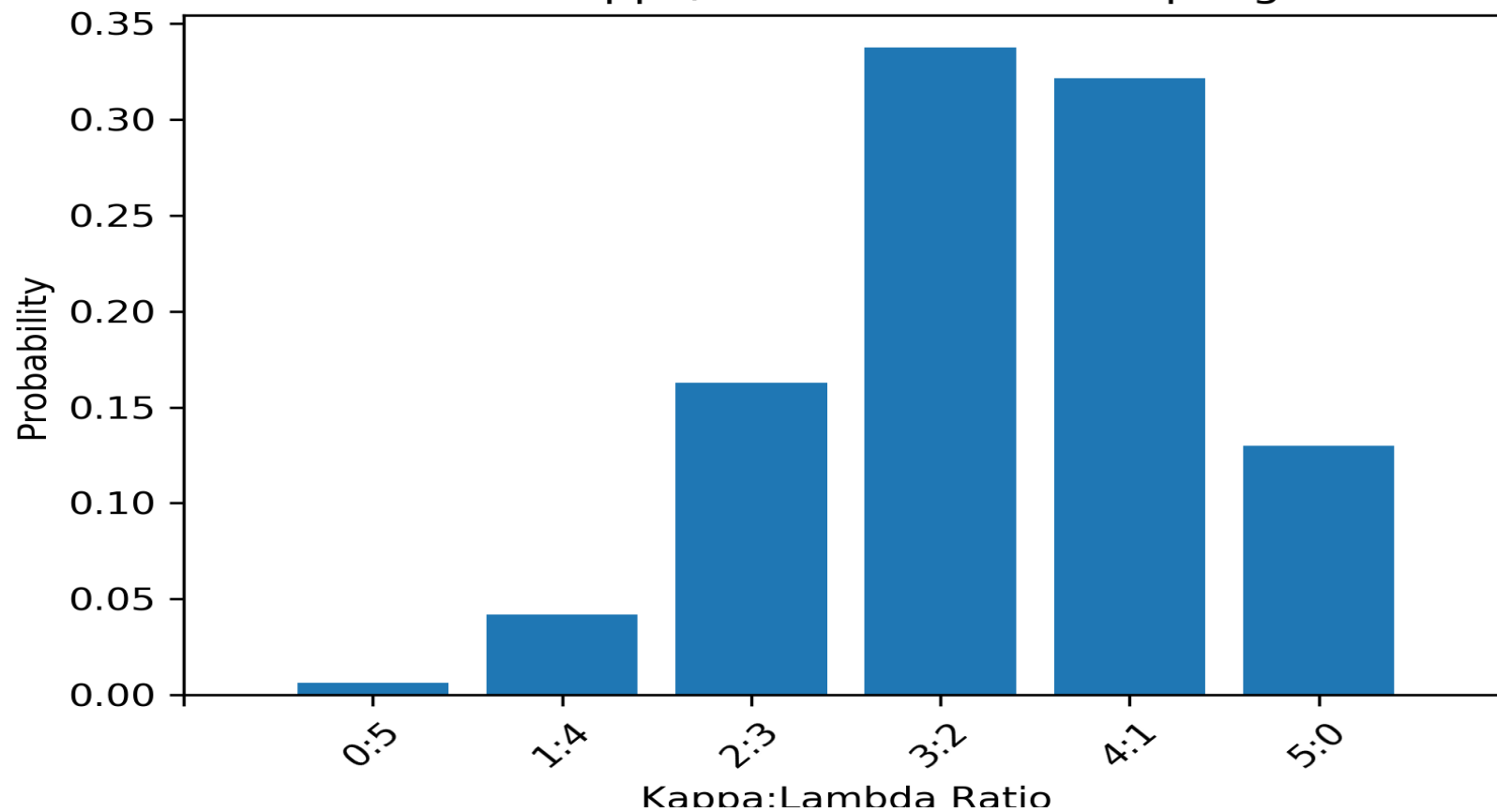
62:37 ~ 3:2



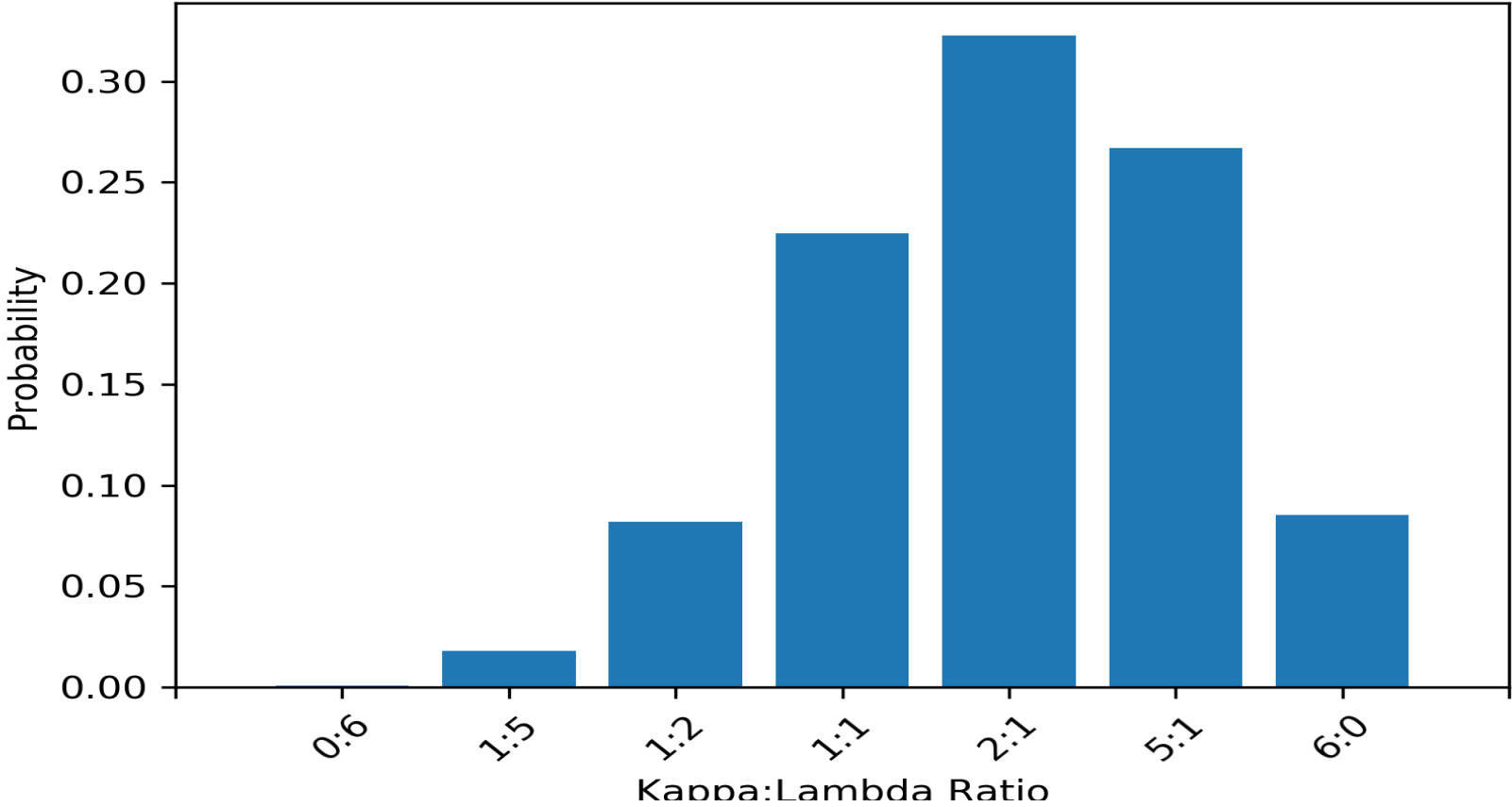
So how many events are enough?



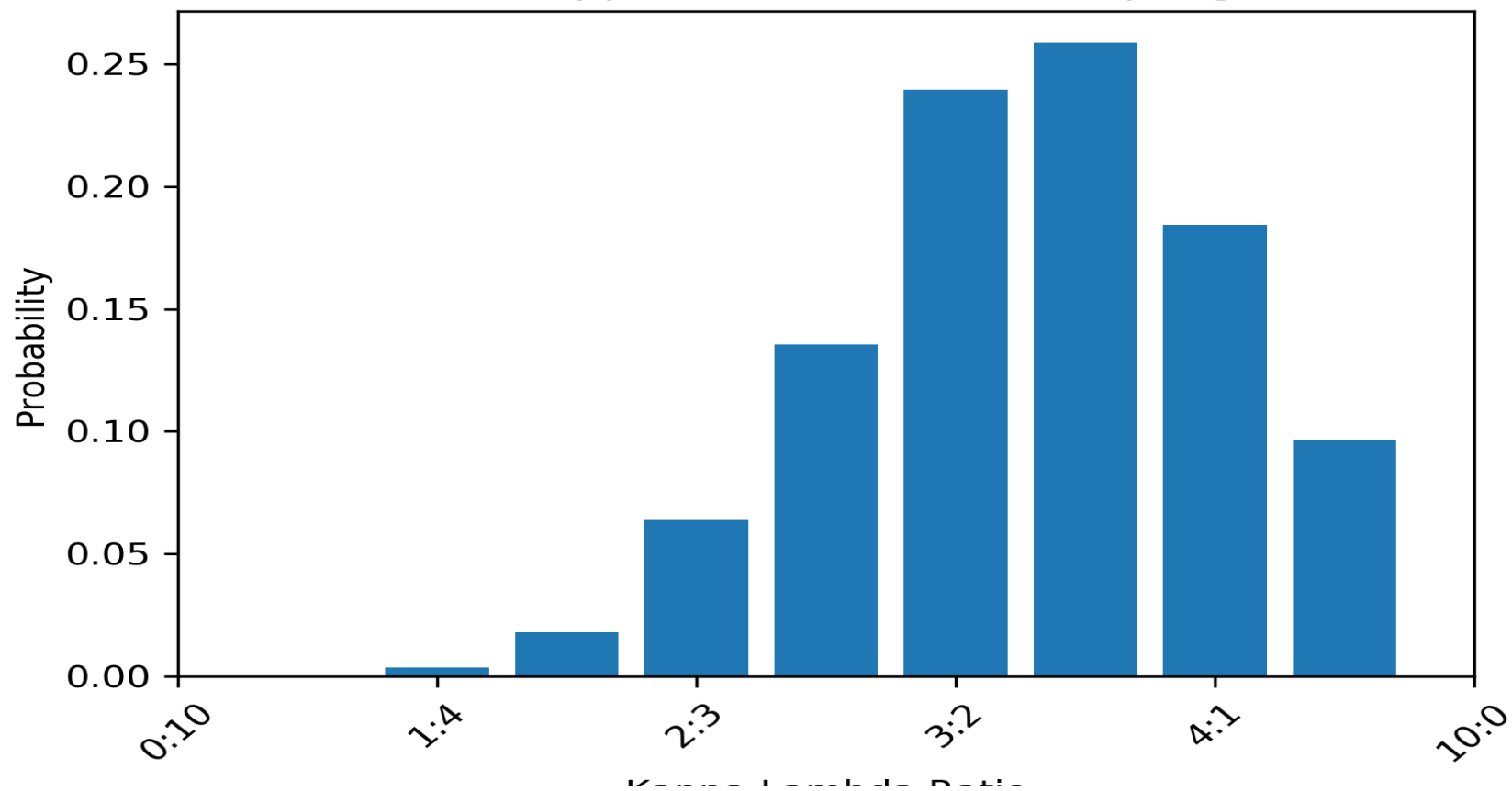
Distribution of Kappa/Lambda ratios sampling 5 events



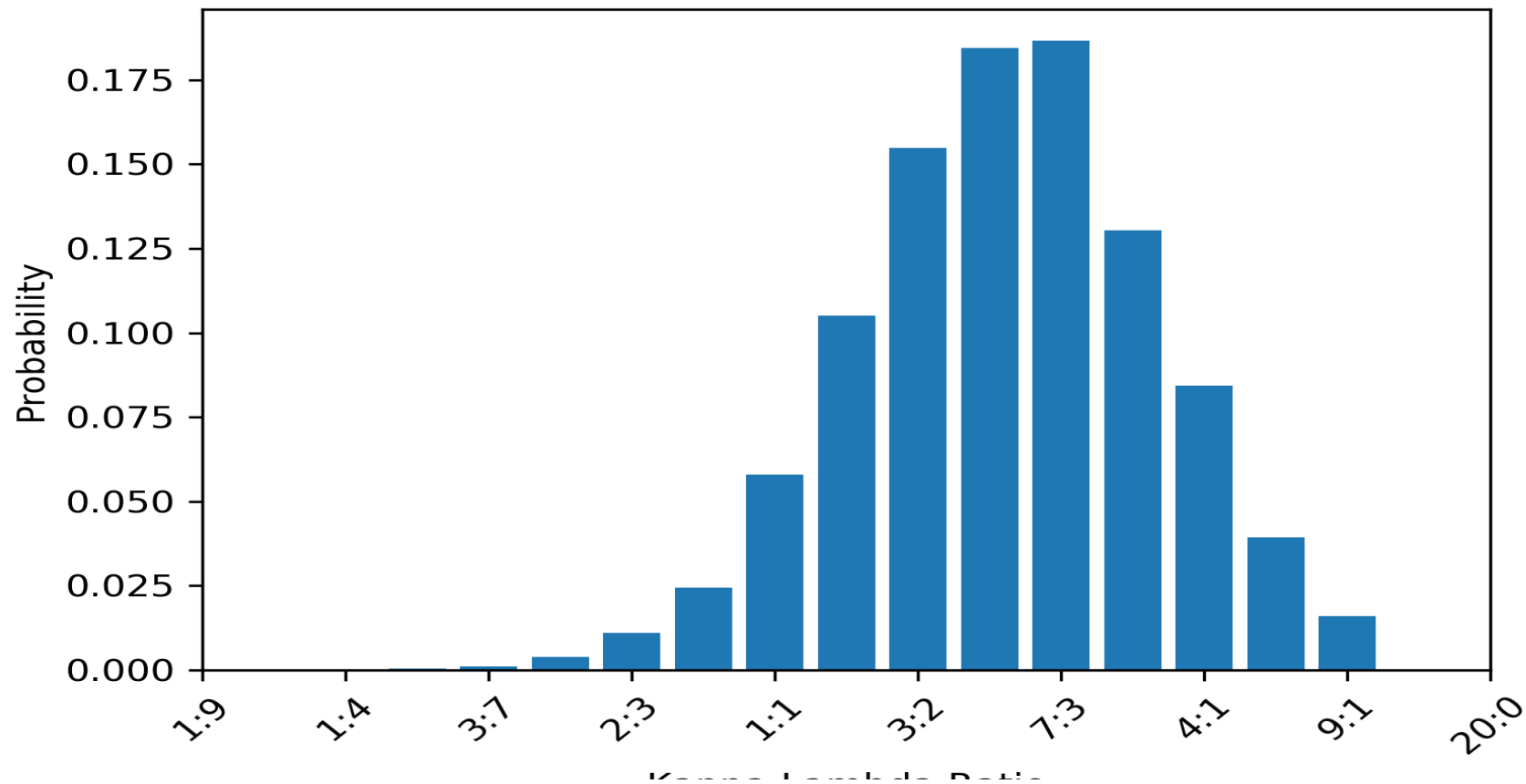
Distribution of Kappa/Lambda ratios sampling 6 events



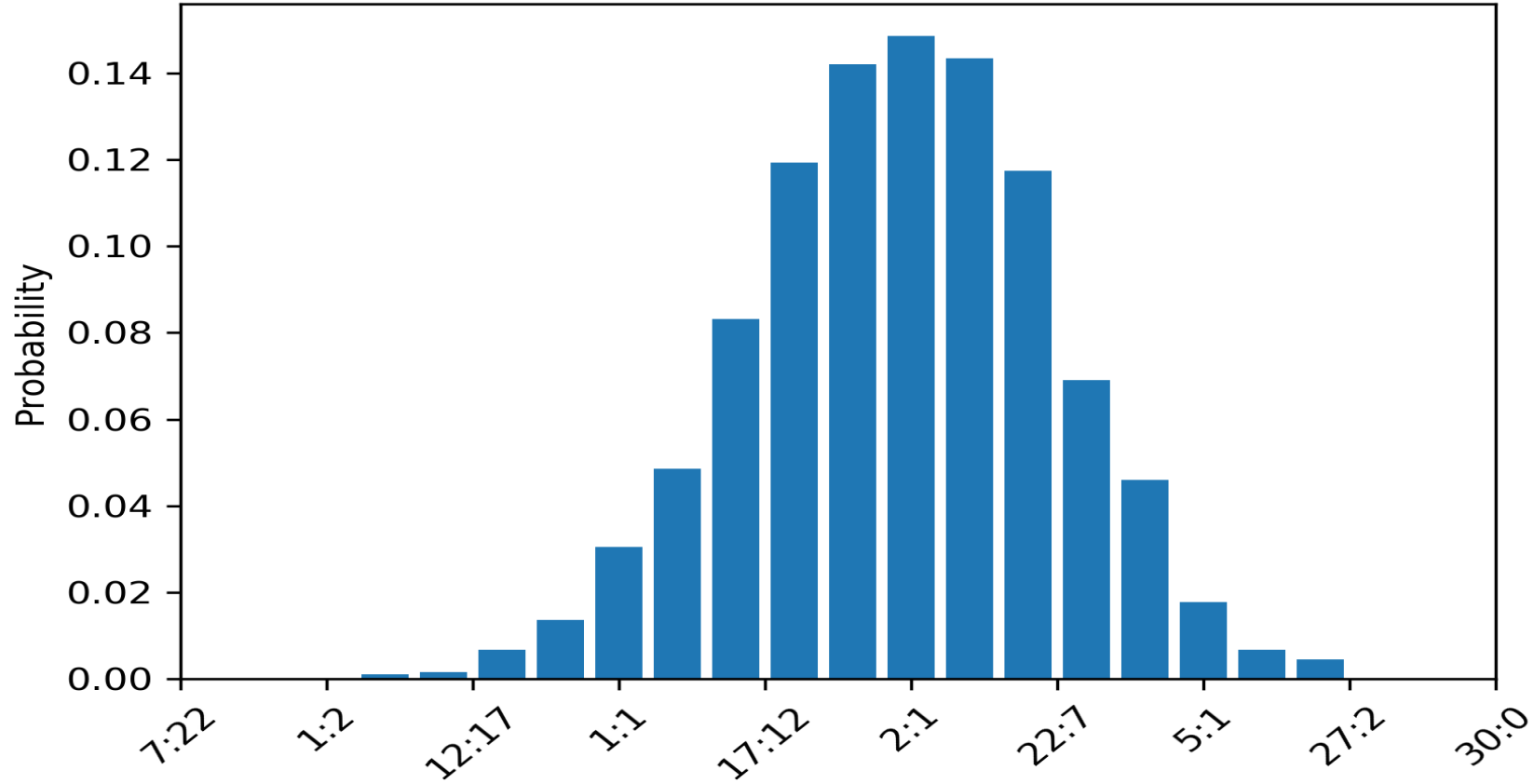
Distribution of Kappa/Lambda ratios sampling 10 events



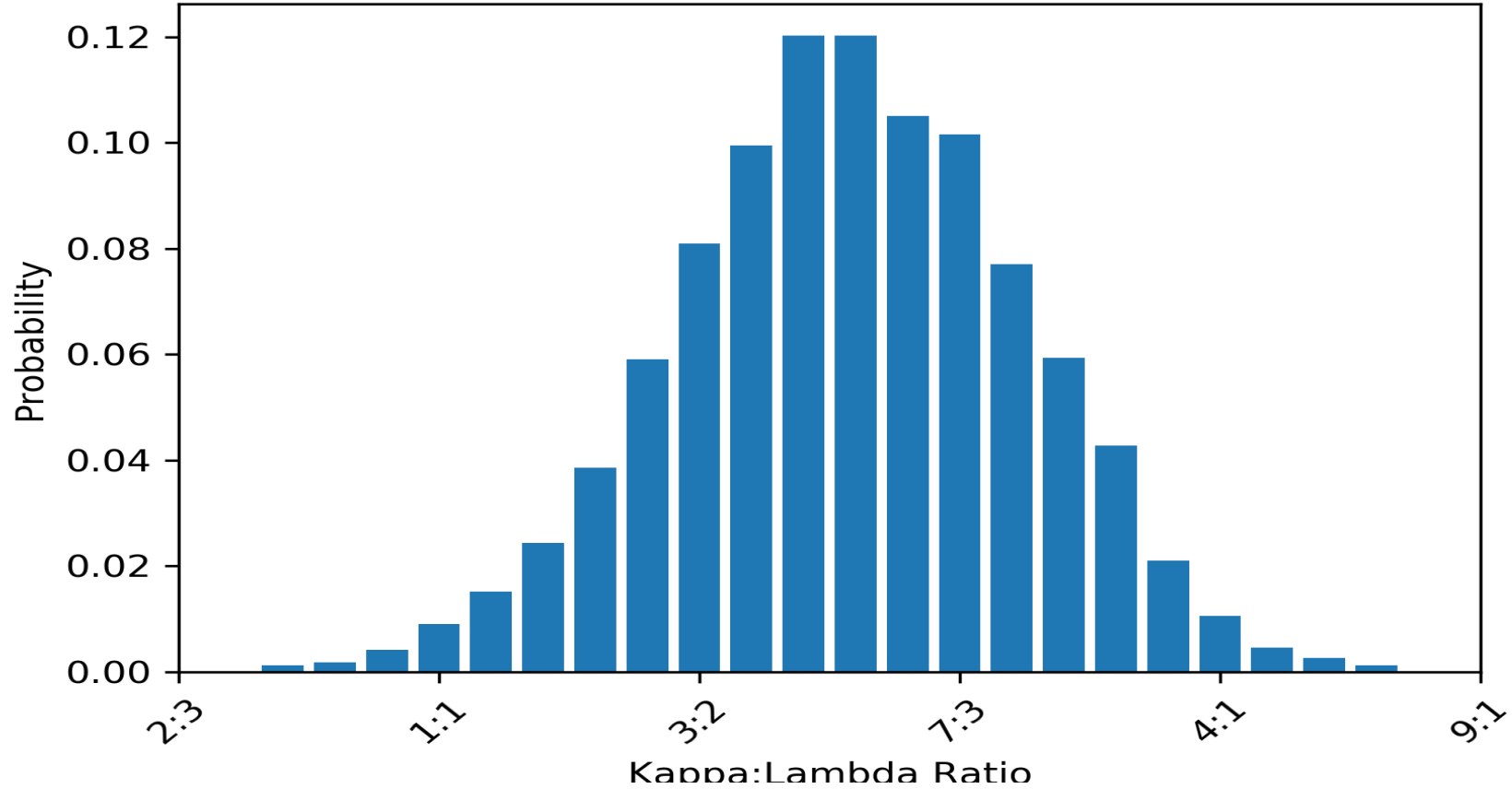
Distribution of Kappa/Lambda ratios sampling 20 events



Distribution of Kappa/Lambda ratios sampling 30 events



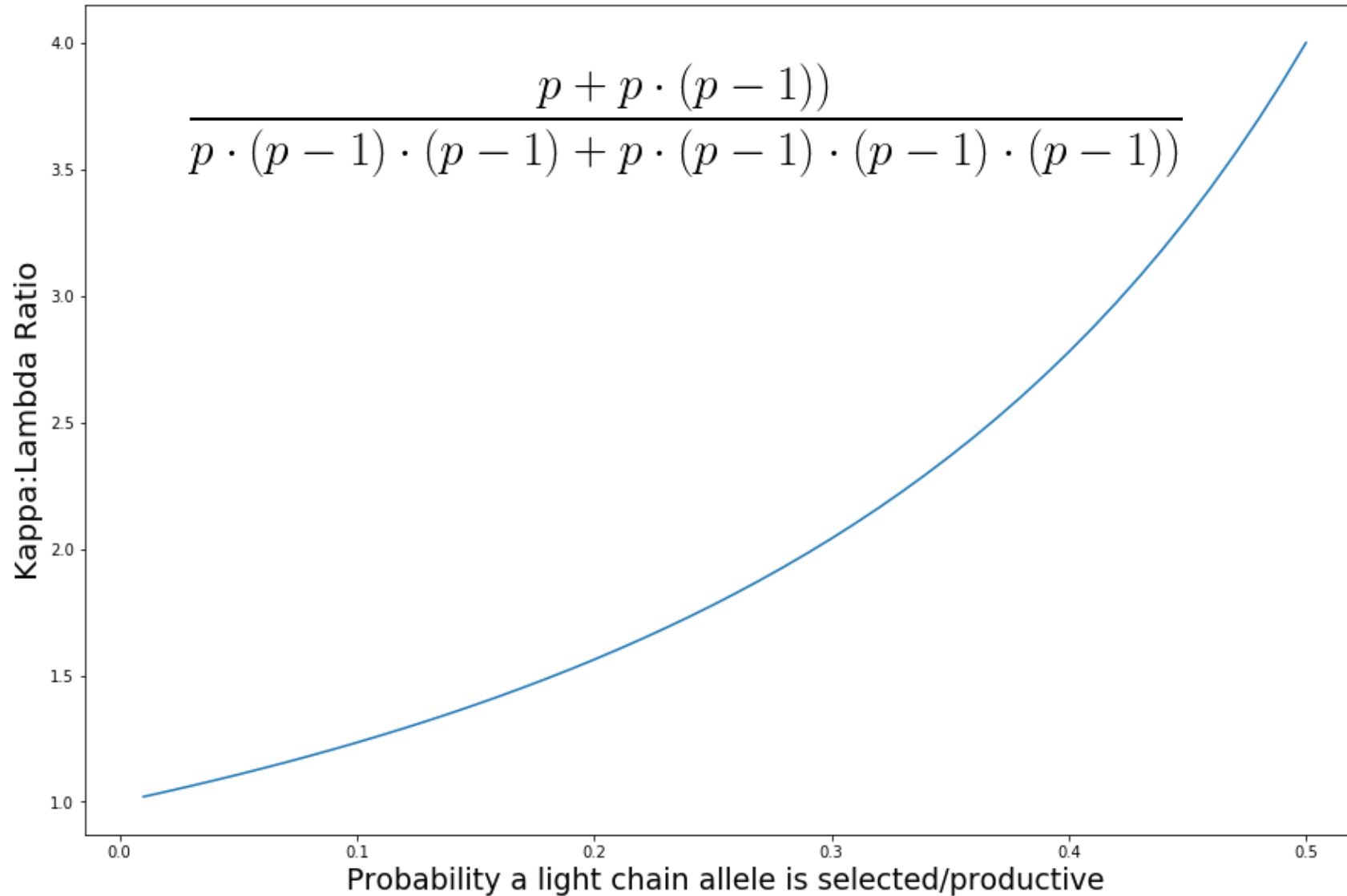
Distribution of Kappa/Lambda ratios sampling 50 events



What about abnormal cases?

- What is a normal range for kappa:lambda ratios?
- 4:1 to 1:2?

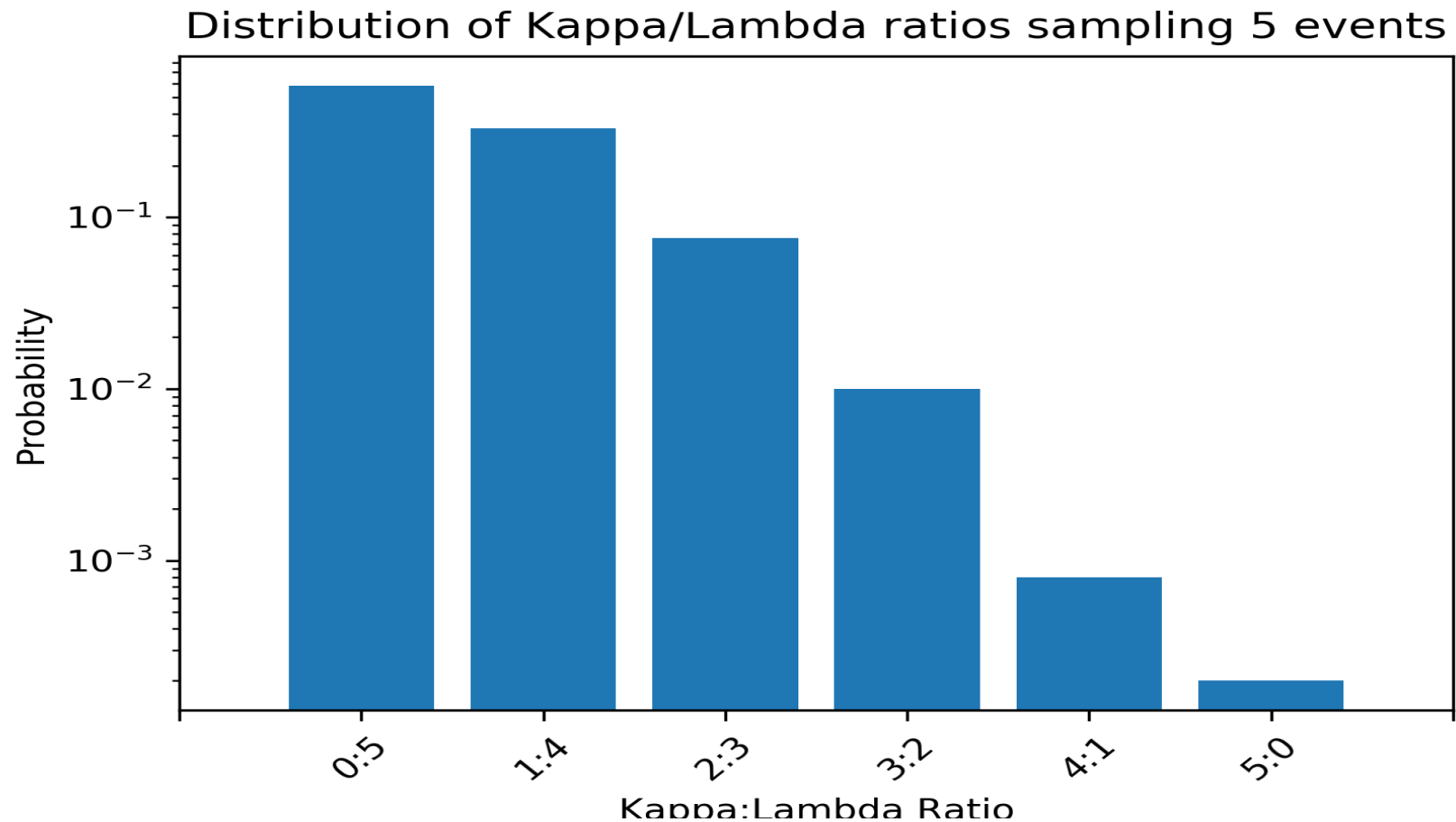
Normal Range of Kappa Lambda?



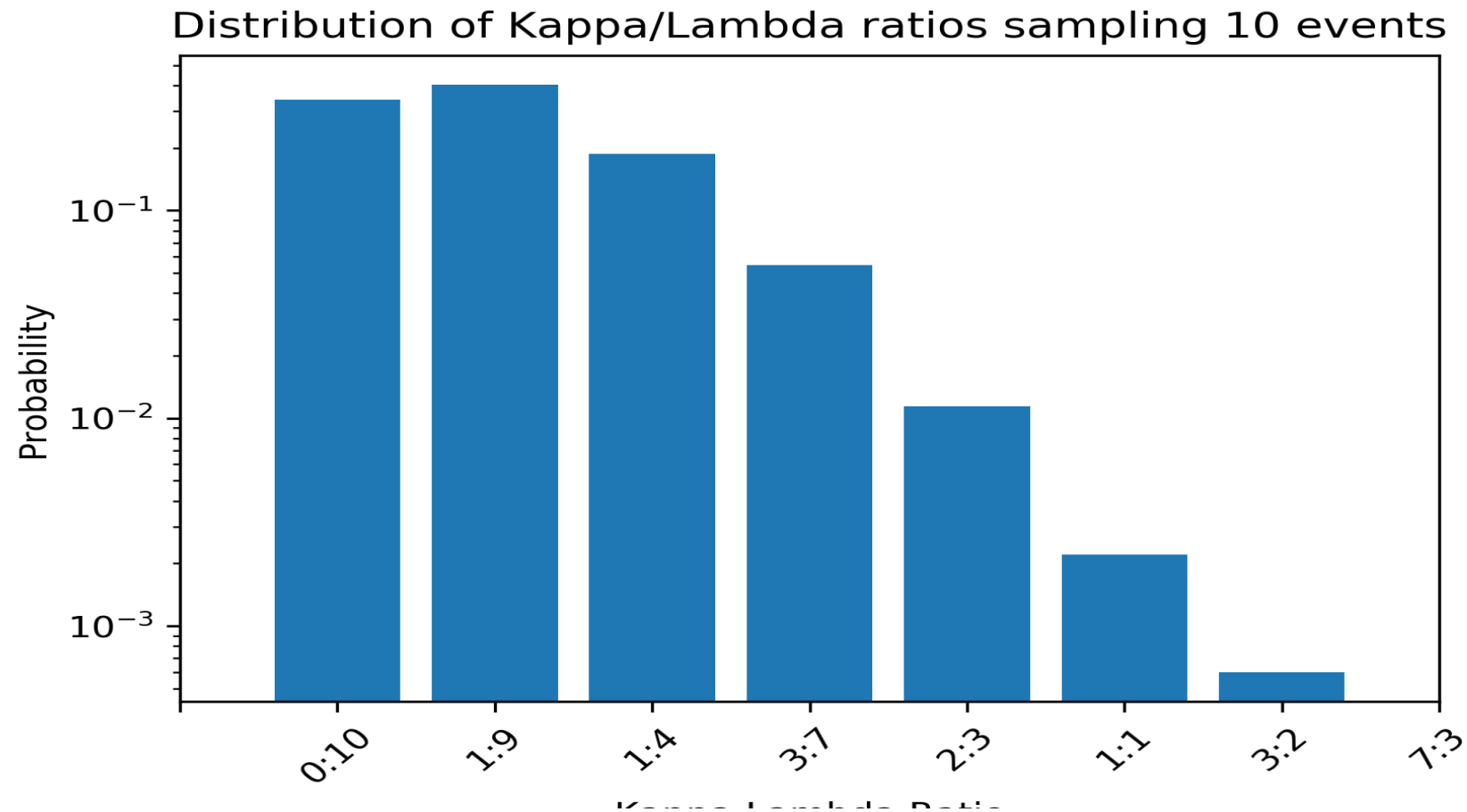
What about abnormal cases?

- What is a normal range for kappa:lambda ratios?
- 4:1 to 1:2?
- What kappa lambda ratios are associated with a malignancy?

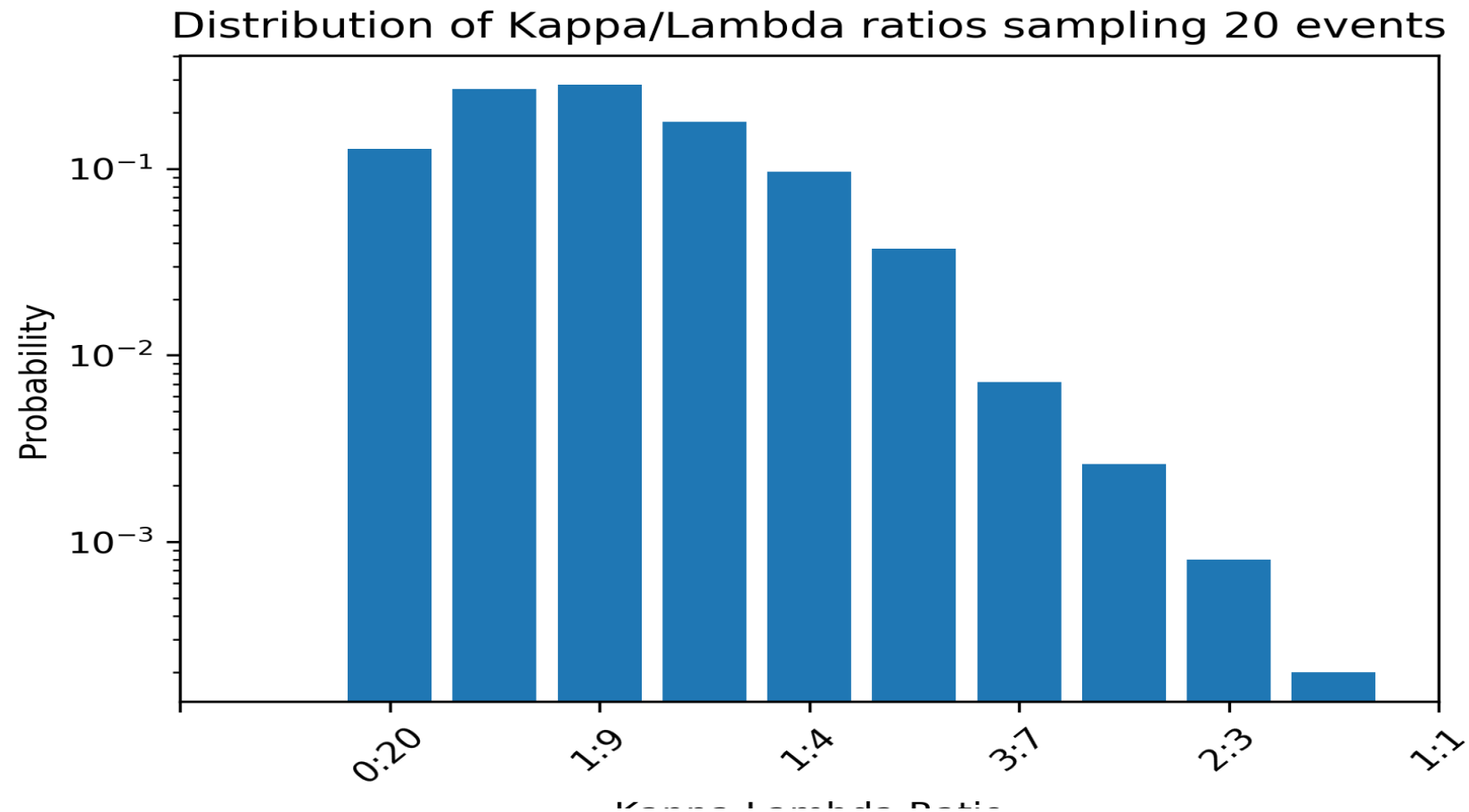
Kappa:Lambda ratio of 1:9



Kappa:Lambda ratio of 1:9

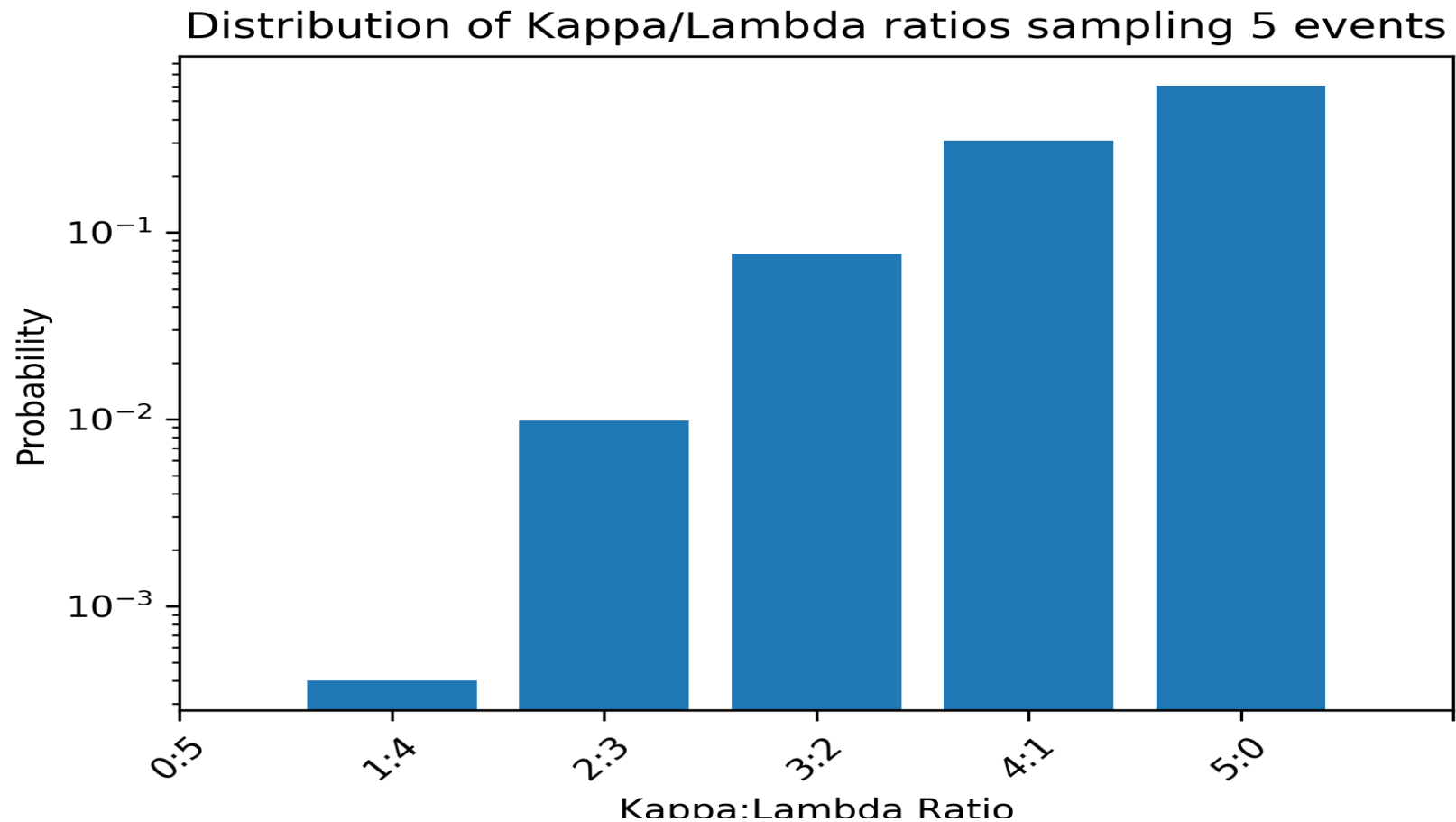


Kappa:Lambda ratio of 1:9

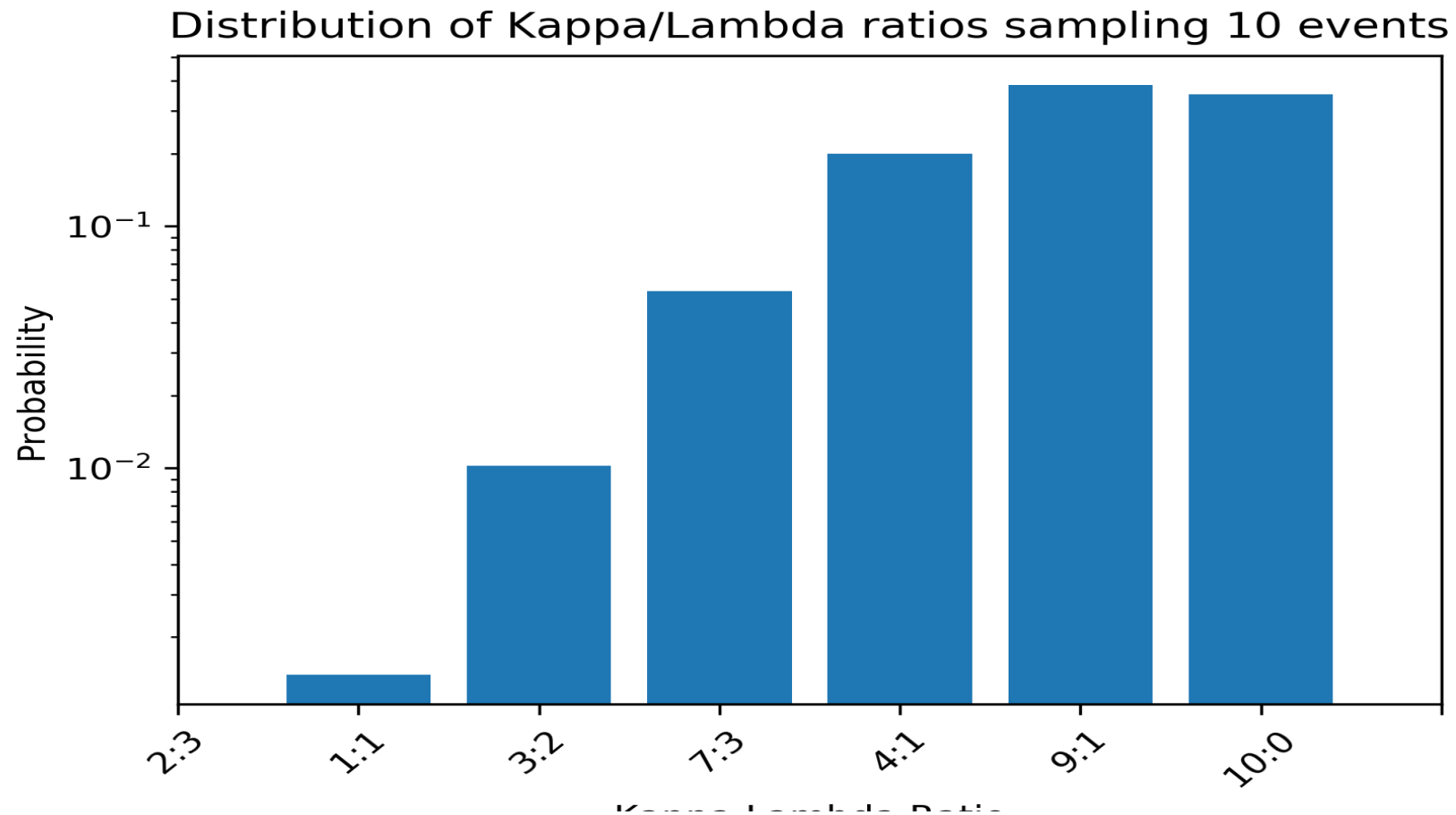


Seems great right?

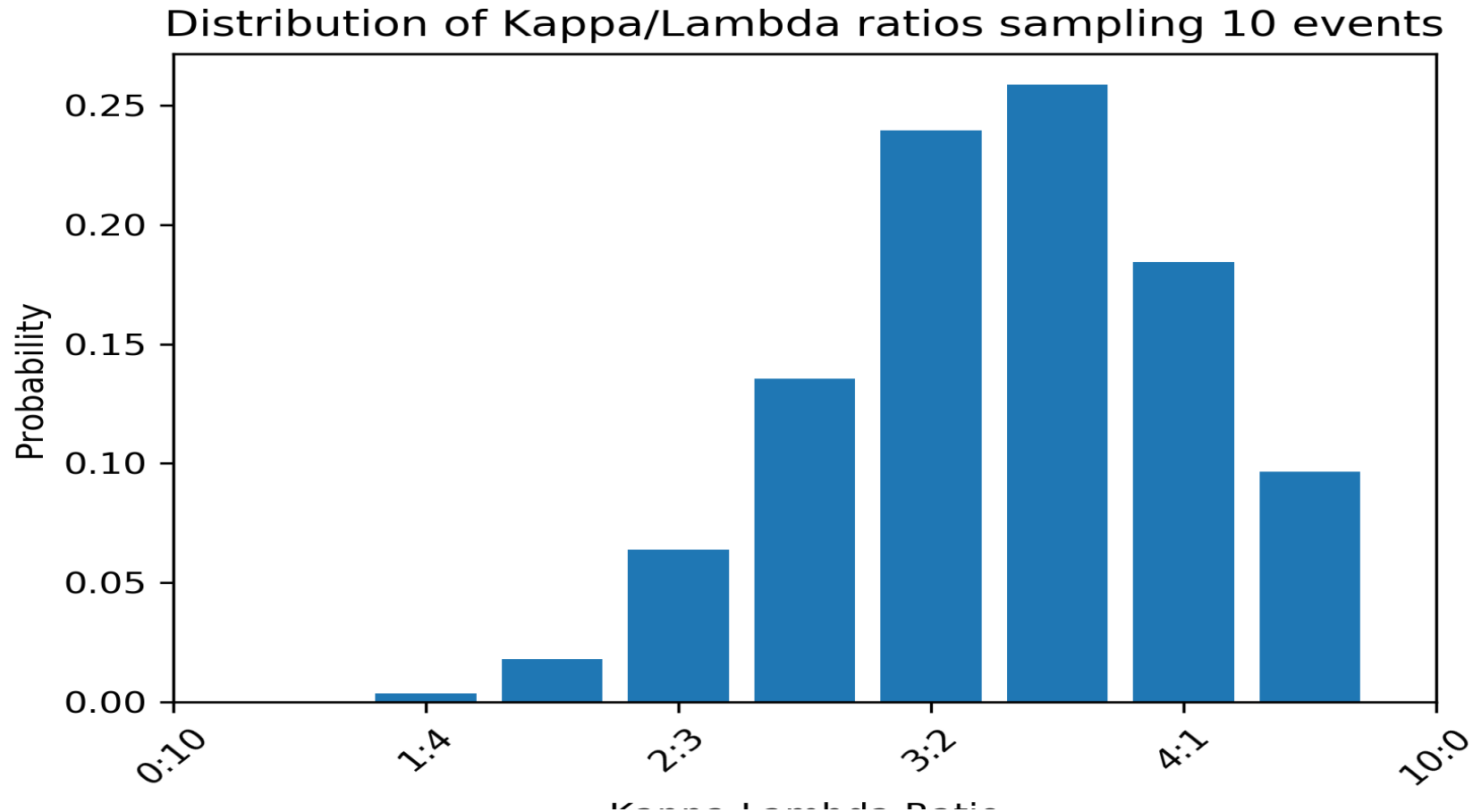
Kappa:Lambda ratio of 9:1



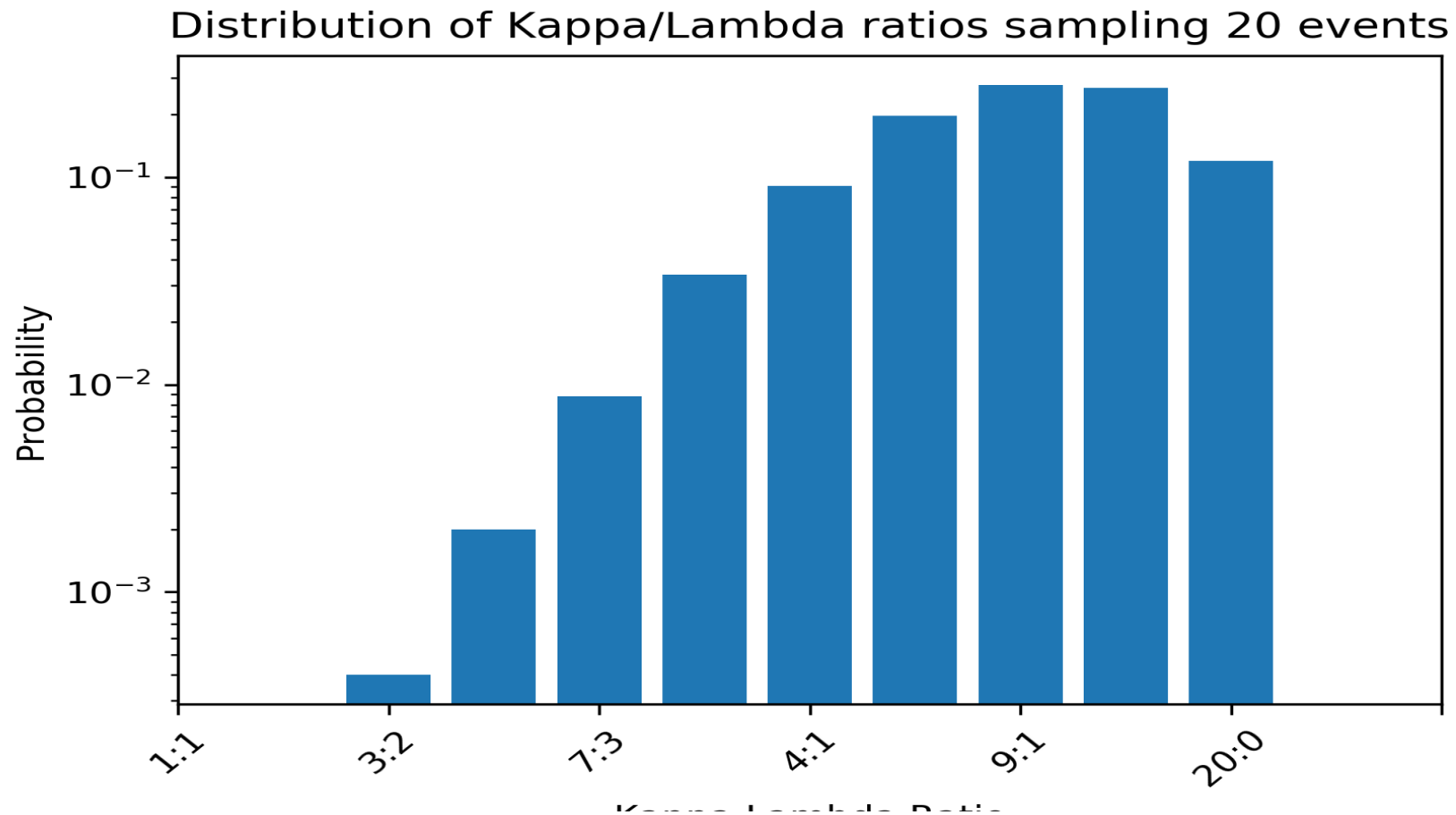
Kappa:Lambda ratio of 9:1



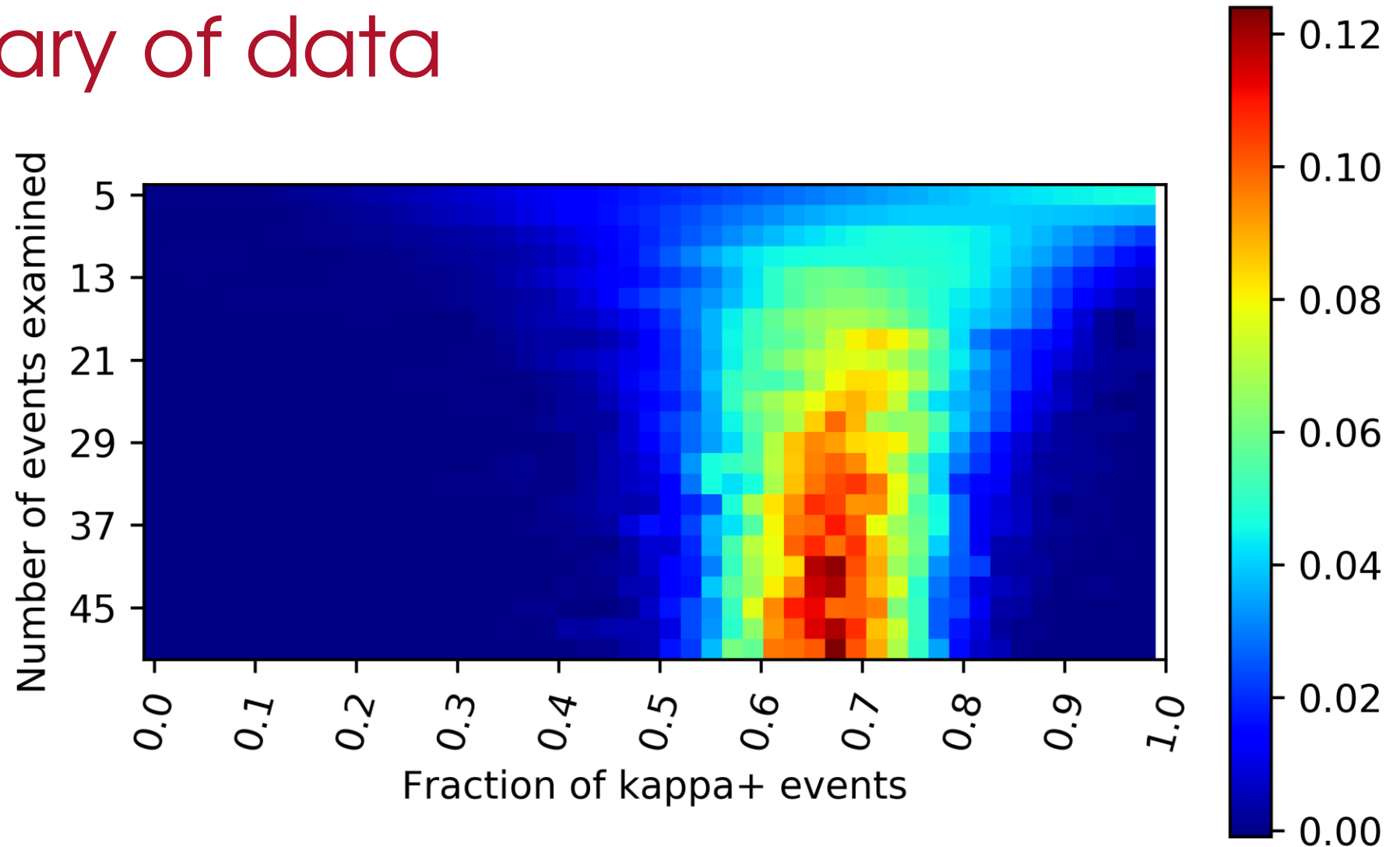
What if the null hypothesis is true?



Kappa:Lambda ratio of 9:1



Summary of data



How about MRD testing?

- How many events are enough to make a call?
- Lets begin with the assumptions
- There may be a lower level of detection caused by:
 - » Artefactual events
 - » Real events

Poisson Distribution



- “a discrete probability distribution that expresses the probability of a given number of events occurring in a fixed interval of time or space if these events occur with a known constant rate and independently of the time since the last event.”
- Sounds like a problem in flow cytometry...

Poisson Distribution

- Equation (λ number of expected events, k number of total events)

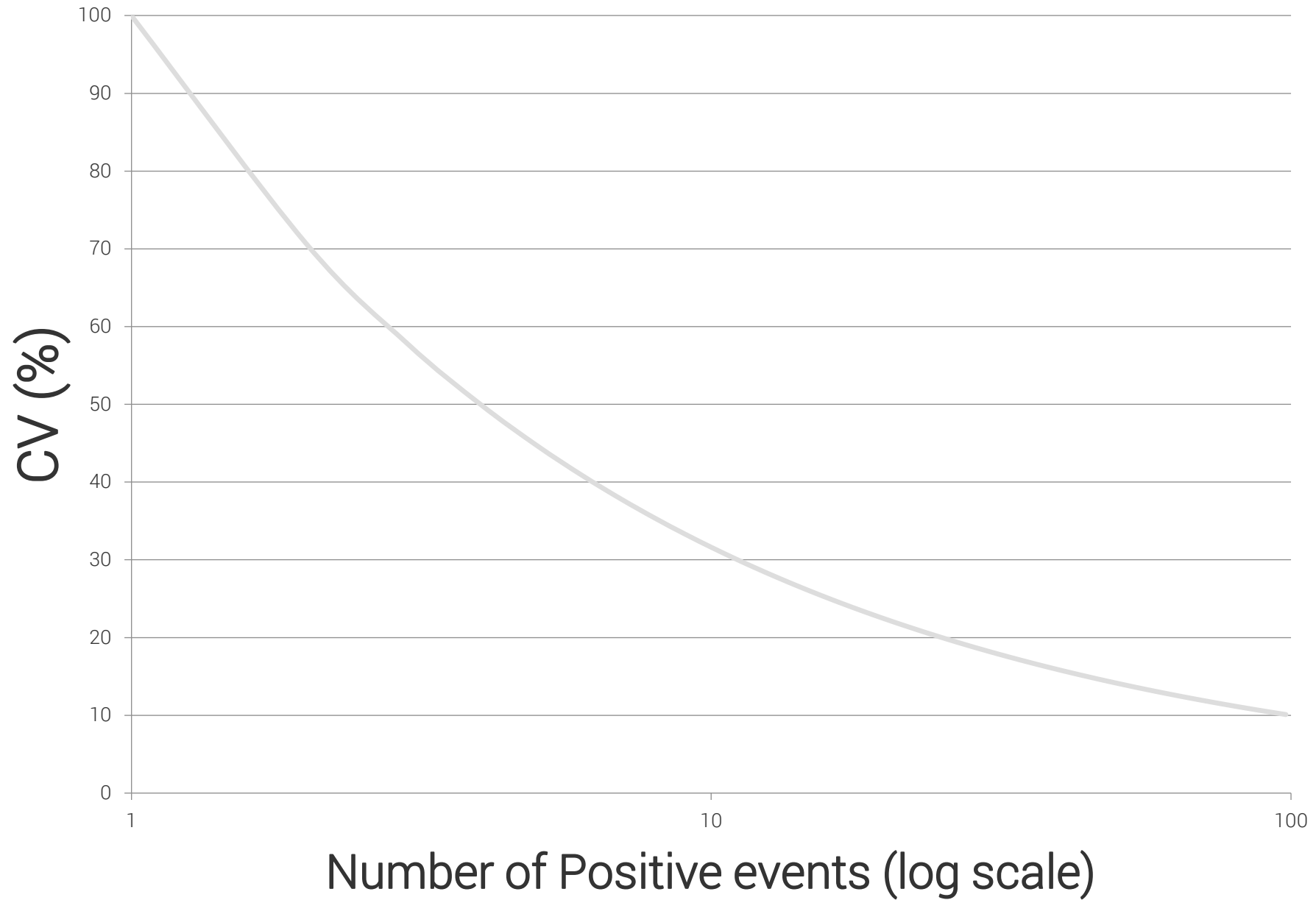
$$P(k \text{ events in interval}) = e^{-\lambda} \frac{\lambda^k}{k!}$$

- Standard Deviation
 - » Equal to the square root of the number of expected events $\sqrt{\lambda}$
- CV
 - » $\lambda/\sqrt{\lambda} = \sqrt{\lambda}$

What does this mean?

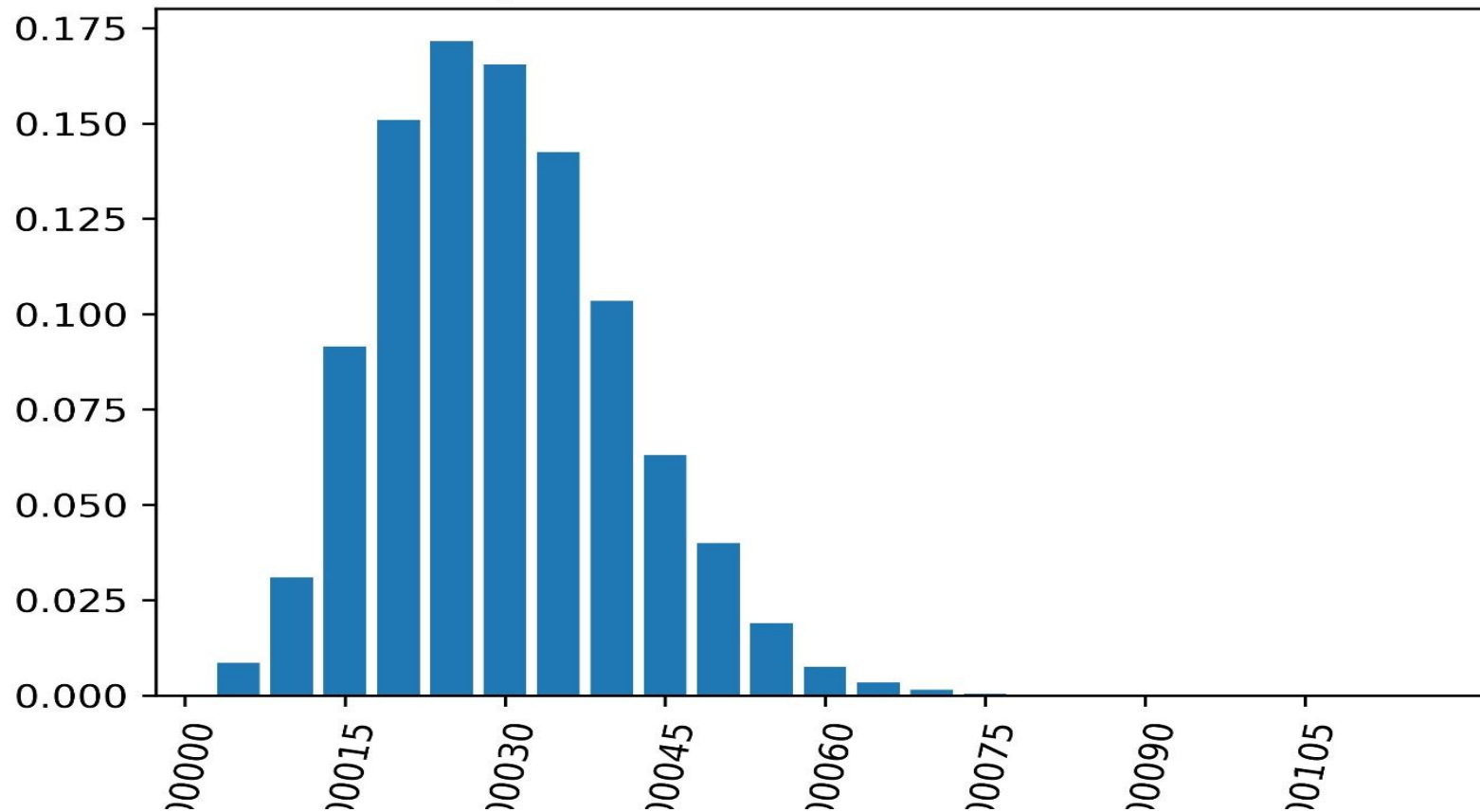
- The CV is inversely proportional to the number of positive events!

Events vs CV (Poisson Distribution)



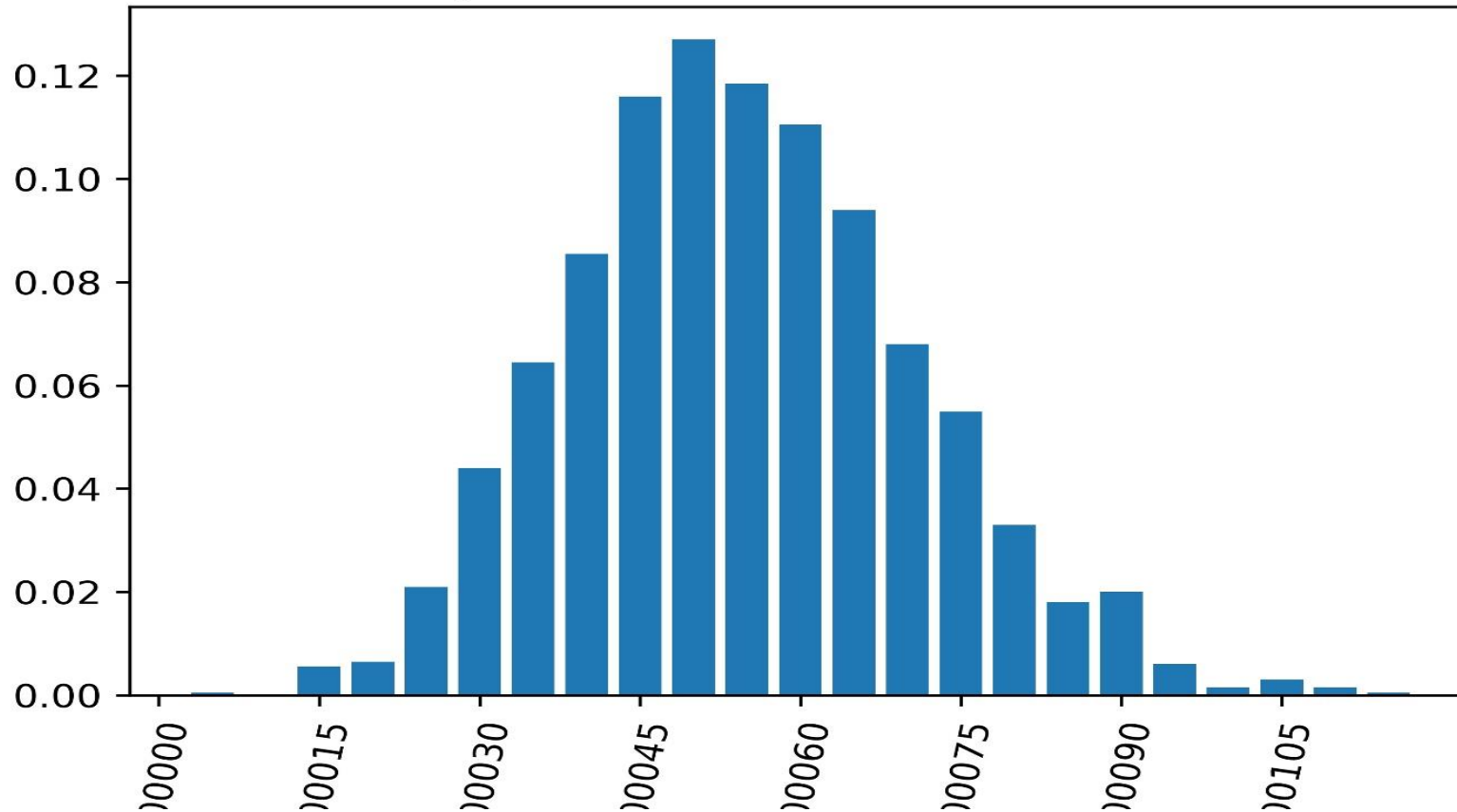
Background

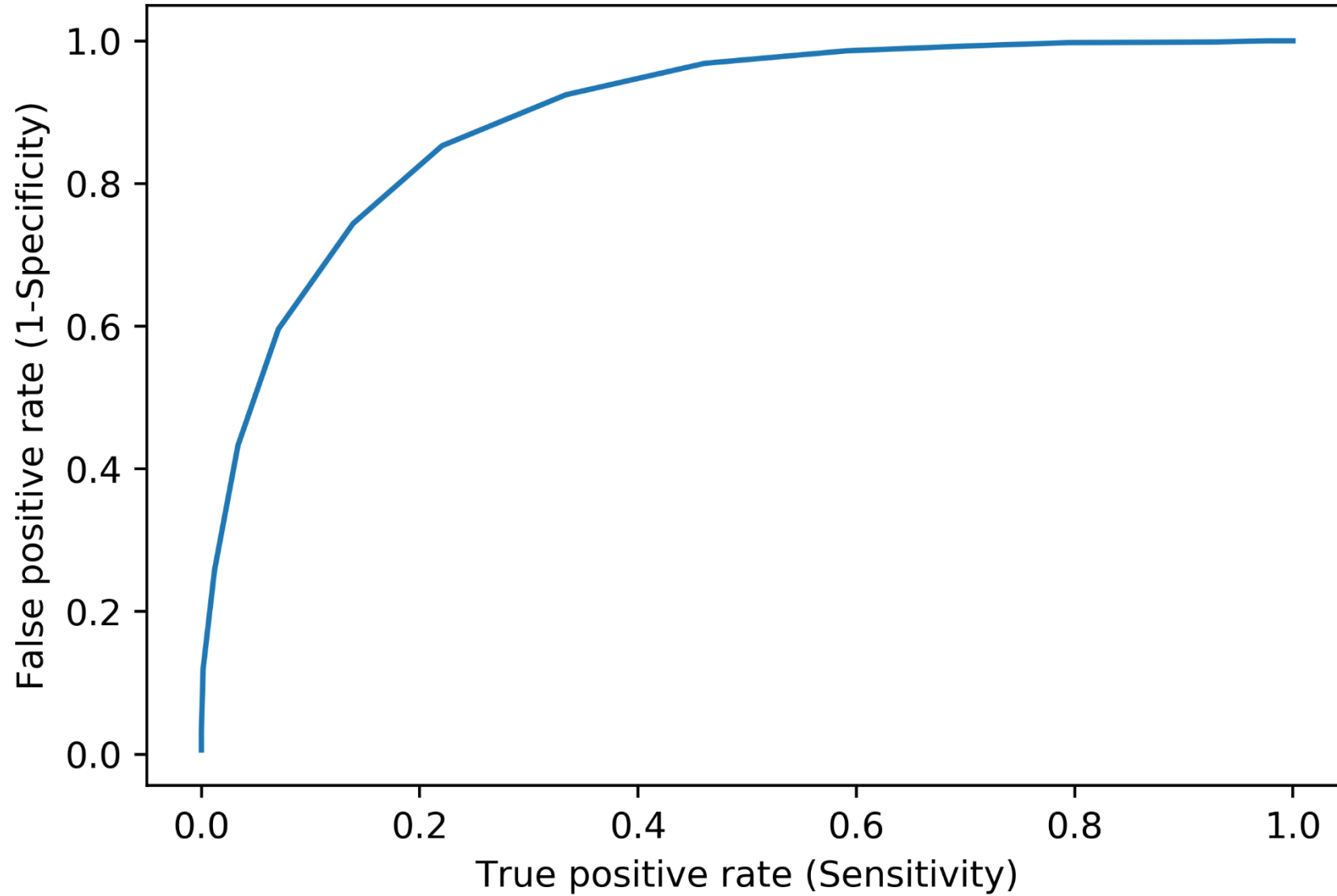
Plot of number of events vs probability
for 0.0025 percent level and 200000 total events



Tumor

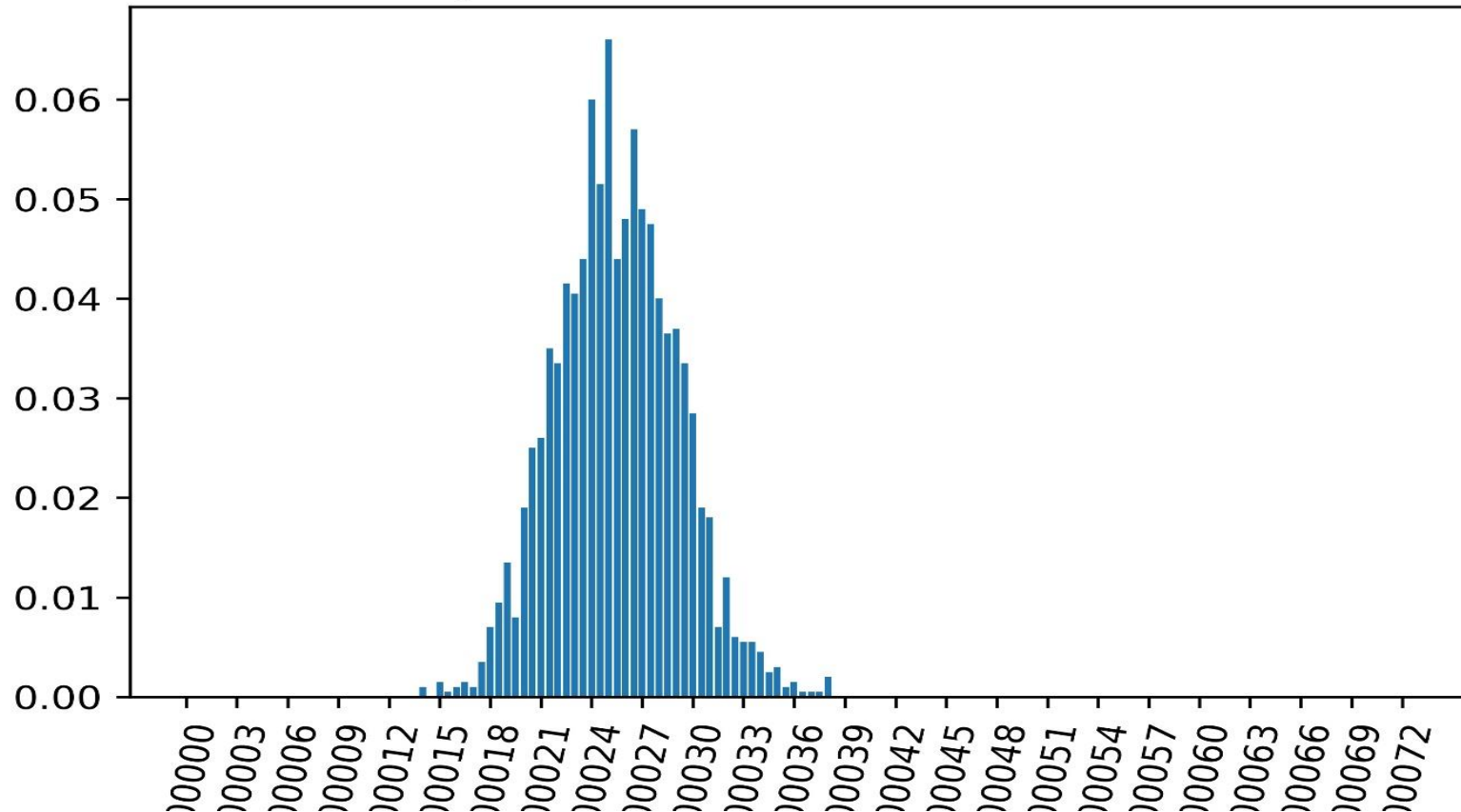
Plot of number of events vs probability
for 0.005 percent level and 200000 total events



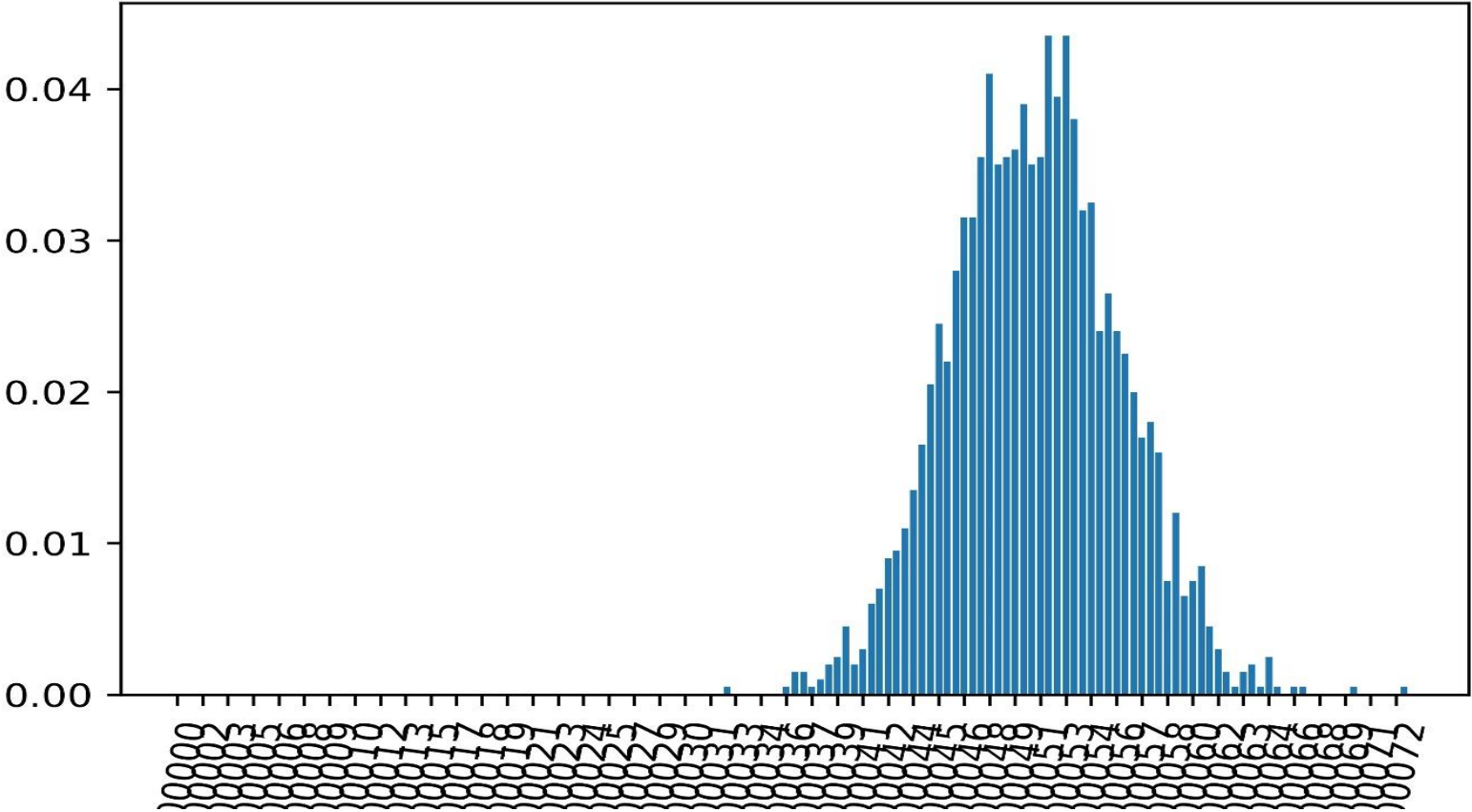


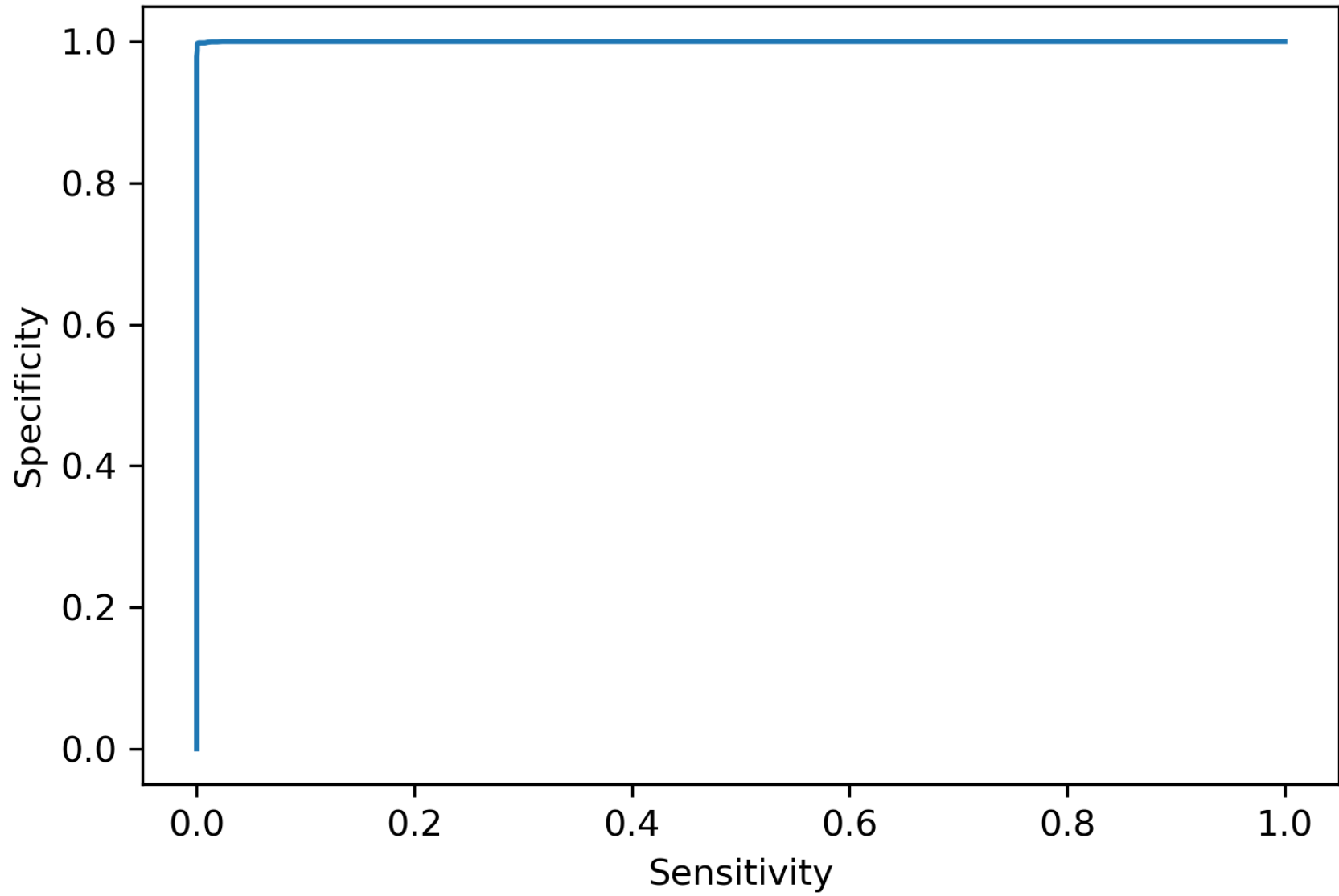
More events?

Plot of number of events vs probability
for 0.0025 percent level and 2000000 total events



Plot of number of events vs probability
for 0.005 percent level and 2000000 total events





More events is 'better'

- It does NOT increase your lower limit of detection
- It does get you closer to your lower limit of detection
- The commonly quoted 50 event criteria is conservative
has an average CV of 14% or the 95% CI is +/-14

Limit of Blank/Detection/Quant

- LOB – limit of blank
 - » $\text{mean}_{\text{blank}} + 1.645(\text{SD}_{\text{blank}})$
 - » Highest apparent analyte concentration in replicates of a blank
- LOD – limit of detection
 - » $\text{LoB} + 1.645(\text{SD}_{\text{low concentration sample}})$
 - » $\text{mean}_{\text{blank}} + 3(\text{SD}_{\text{blank}})$
 - » 20 events is a quoted standard
 - » lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible
- LOQ – limit of quantification
 - » 50 events is a 'standard'

What is an abnormal cell?

- 10c flow buys you multiple aberrancies on a population
- Typically want 2+ aberrancies to call
- Some aberrancies are better than others

Examples

- Myeloma MRD
- B-LBL MRD
- CLL MRD
- AML MRD...

Consensus Guidelines on Plasma Cell Myeloma Minimal Residual Disease Analysis and Reporting

Maria Arroz,^{1*} Neil Came,² Pei Lin,³ Weina Chen,⁴ Constance Yuan,⁵ Anand Lagoo,⁶
Mariela Monreal,⁷ Ruth de Tute,⁸ Jo-Anne Vergilio,⁹ Andy C. Rawstron,¹⁰
and Bruno Paiva¹¹

- Recommended consensus panel: CD38, CD138, CD45, CD56, CD19, CD27, CD81 and CD117, κ and λ optional (does not give additional information in 97% of cases)
- Target a lower limit of detection of 0.001%, which requires 3-5 X 10⁶ events
- Prelysis recommended
Advantages: max number of cells per tube, minimizes antibody use, greater sensitivity
- Recommend analyzing first draw bone marrows
- Highly recommend that laboratories initiating MM MRD testing adopt a validated panel

Most Frequently used Markers for Detection of Myeloma Associated Phenotypes (MAP) Included in MM MRD Panels

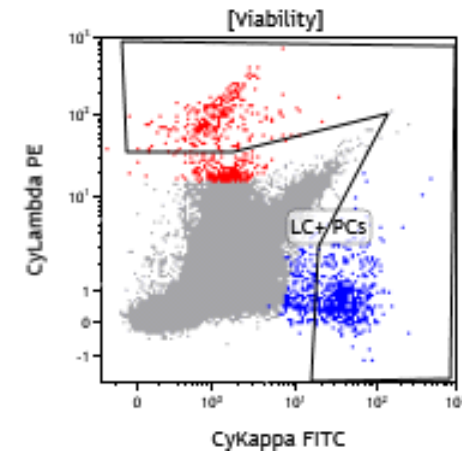
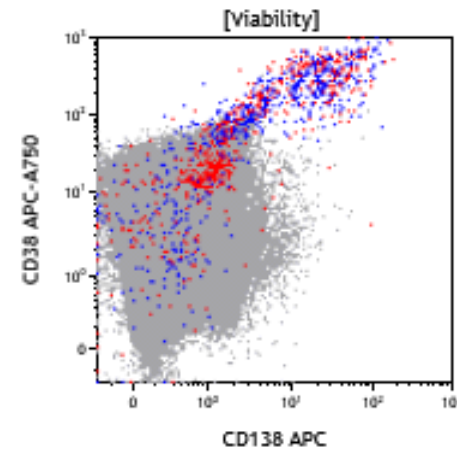
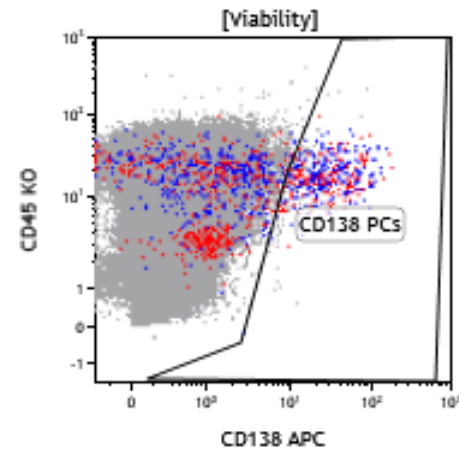
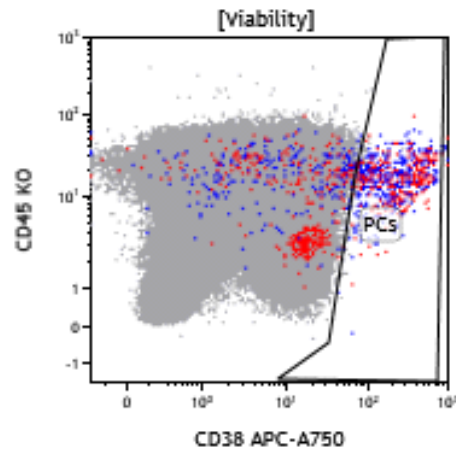
Antigen	Aberrant pattern	% of abnormal expression cases
CD19	-	96%
CD20	dim+	17-30%
CD27	- or dim+	40-68%
CD28	+	15-45%
CD33	+	18%
CD38	dim+	80%
CD45	-	73%
CD54	dim+	60-80%
CD56	++	60-75%
CD81	- or dim+	55%
CD117	+	30-32%
CD200	+ / + +	≥70%
CD307	++	NA

- CD27 is a costimulatory molecule in the TNFr family. Expressed brightly on PCs. Tends to be negative if CD19 is negative (though not vice-versa).
- CD81 forms a signal transduction complex with CD19 on B cells. Expressed brightly on PCs.

ARUP MM MRD Assay

- 5 M events
- 0.001% LOD (50 events lab standard) = LOQ
 - » Need not be the case...
- CD45
- CD19
- CD56
- CD117
- CD27
- CD81

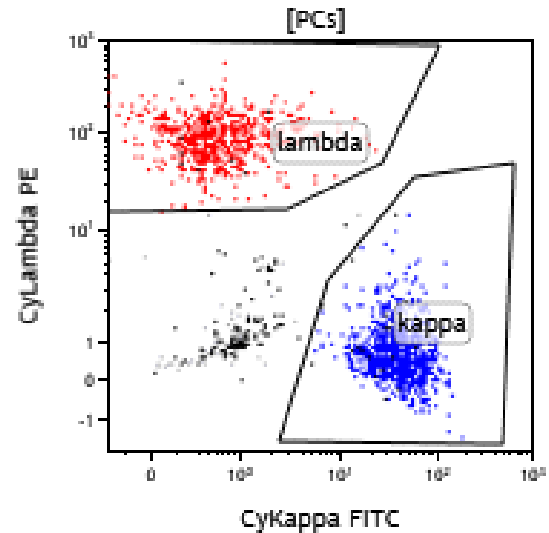
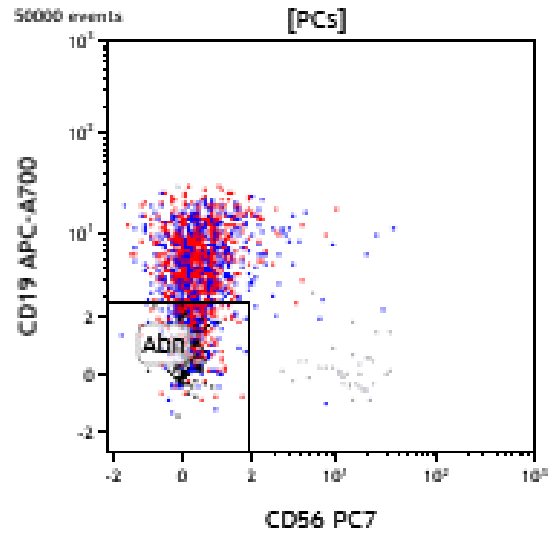
Many ways to find PCs



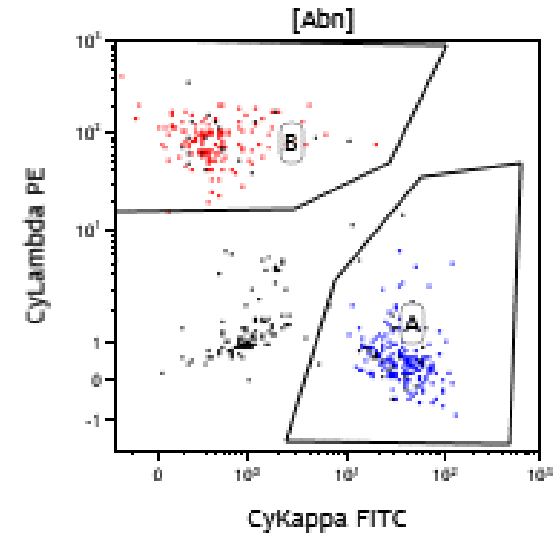
What is the range of normal?

- The world looks different at 2x vs 100x
- 100,000 events vs 5,000,000 events

CD19/CD56

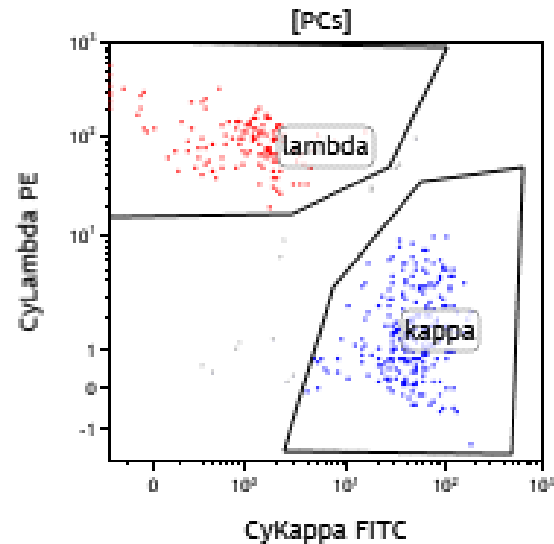
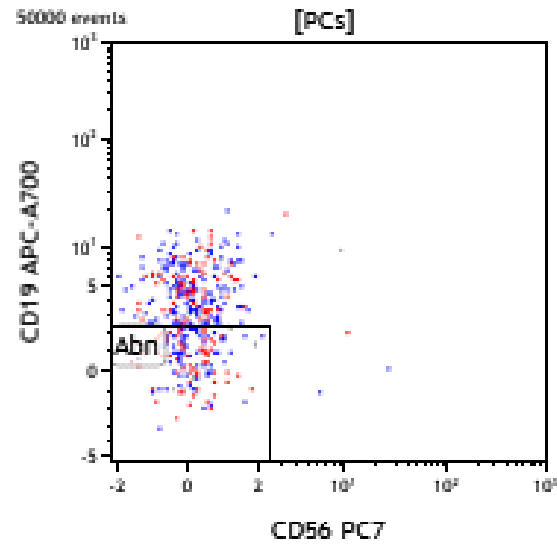


Gate	Number	%Total	%Gated
All	1,736	0.10	100.00
kappa	921	0.05	53.05
lambda	651	0.04	37.50

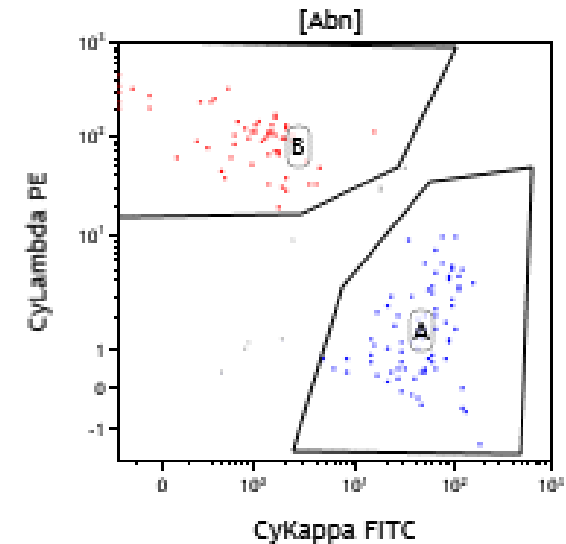


Gate	Number	%Total	%Gated
All	461	0.03	100.00
A	202	0.01	43.82
B	158	0.01	34.27

CD19/CD56 Pitfalls

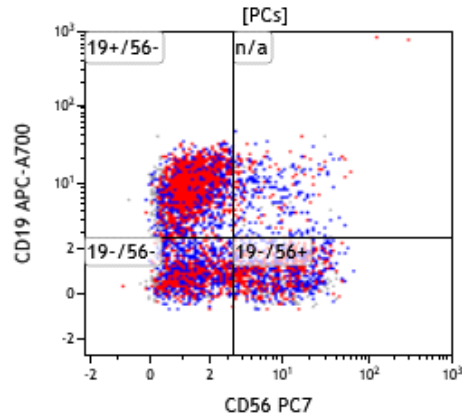


Gate	Number	%Total	%Gated
All	392	0.02	100.00
kappa	243	0.01	61.99
lambda	133	0.01	33.93

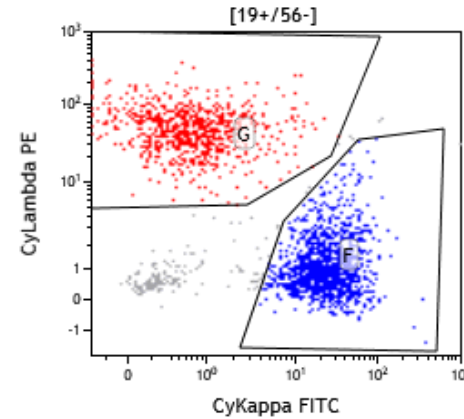


Gate	Number	%Total	%Gated
All	148	0.01	100.00
A	78	0.00	52.70
B	61	0.00	41.22

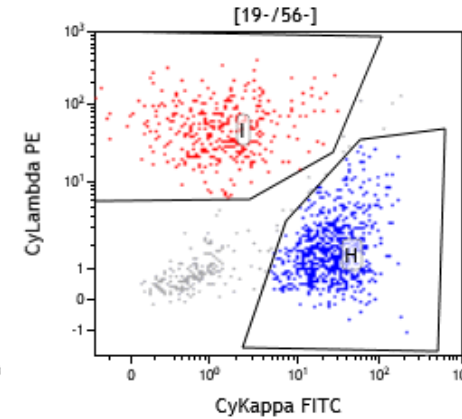
CD19/CD56 Pitfalls



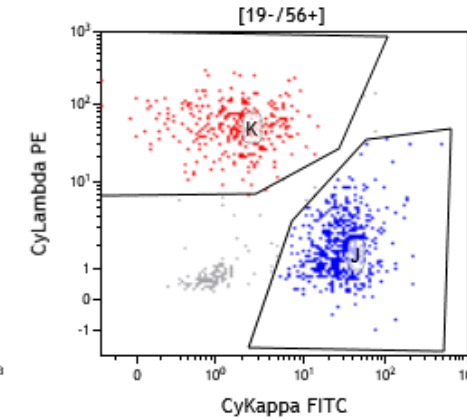
Gate	Number	%Total	%Gated
All	4,770	0.28	100.00
19-/56-	1,222	0.07	25.62
19-/56+	984	0.06	20.63
19+/56-	2,256	0.13	47.30
n/a	308	0.02	6.46



Gate	Number	%Total	%Gated
All	2,256	0.13	100.00
F	1,322	0.08	58.60
G	762	0.04	33.78

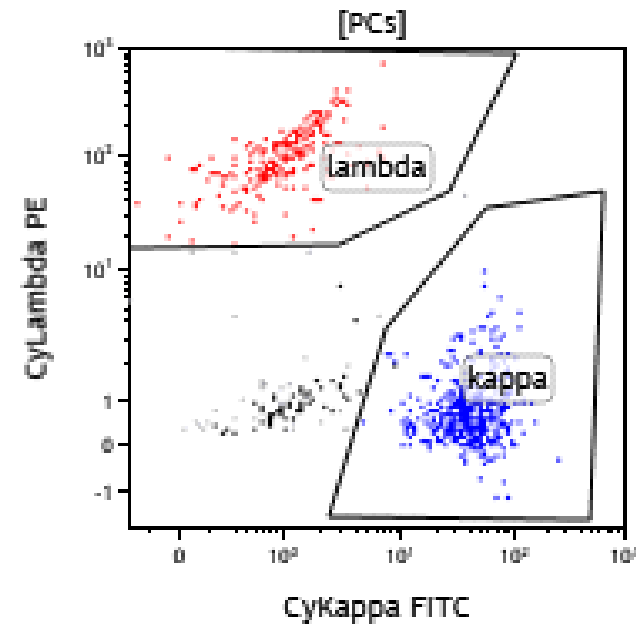
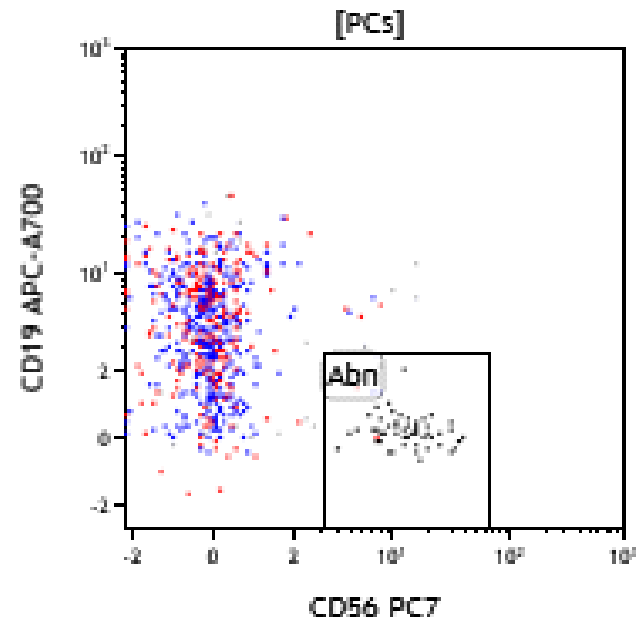


Gate	Number	%Total	%Gated
All	1,222	0.07	100.00
H	692	0.04	56.63
I	347	0.02	28.40



Gate	Number	%Total	%Gated
All	984	0.06	100.00
J	539	0.03	54.78
K	305	0.02	31.00

CD19/CD56 Pitfalls

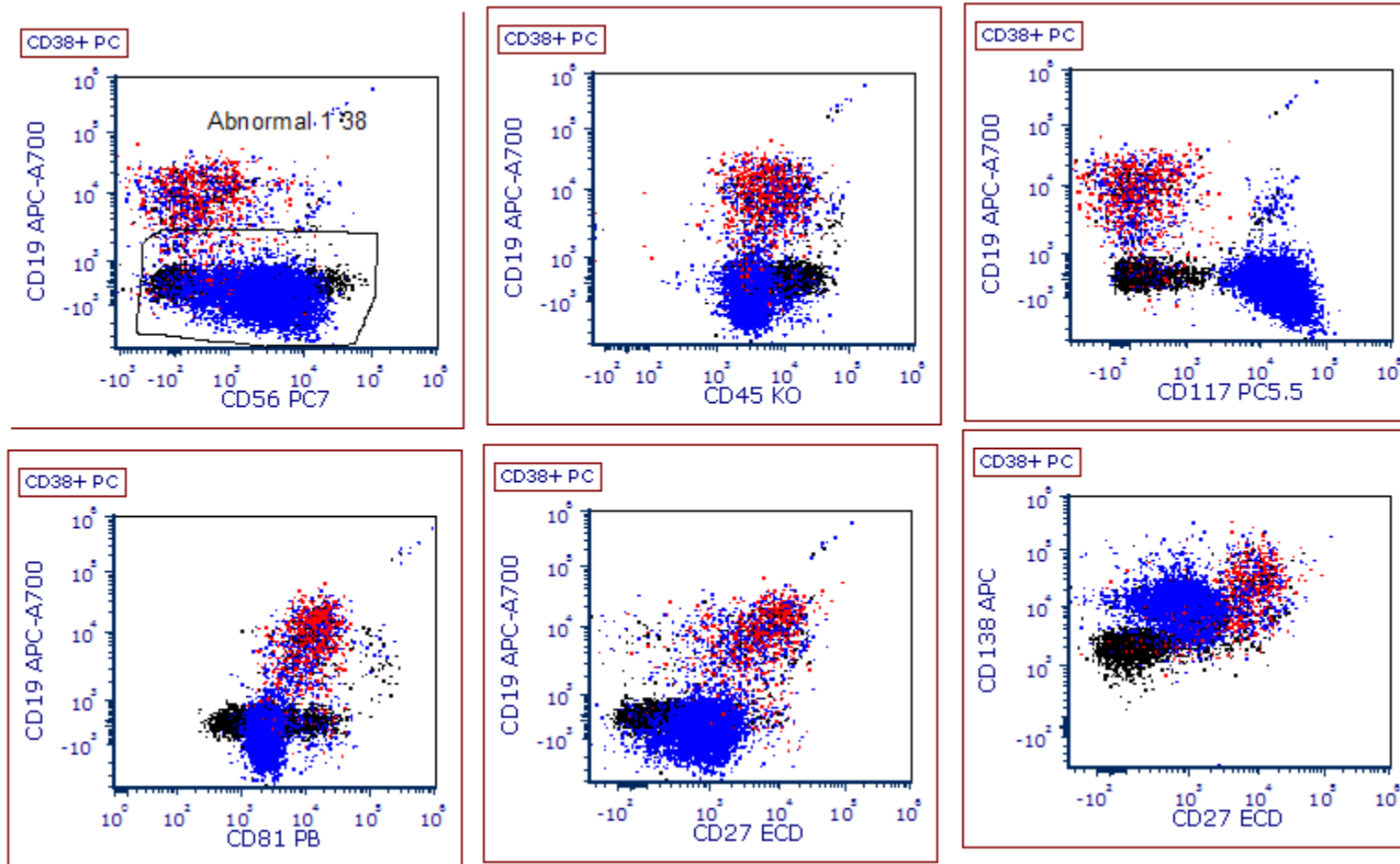


Gate	Number	%Total	%Gated
All	716	0.04	100.00
kappa	409	0.02	57.12
lambda	217	0.01	30.31

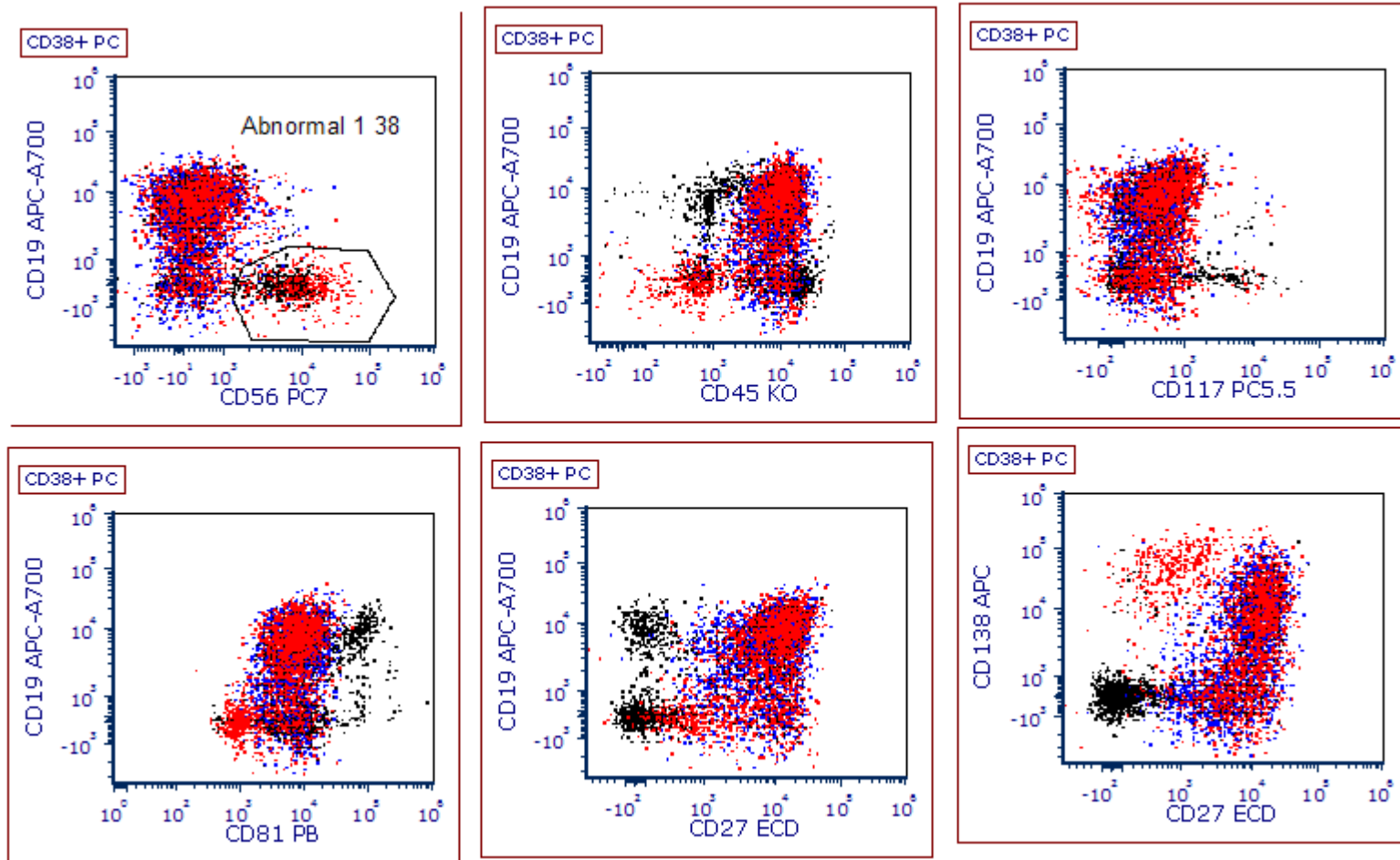
Take home point

- Resident polytypic 19-/56+ populations exist
- CD56+ things fall into the PC gates (activated NK cells?)

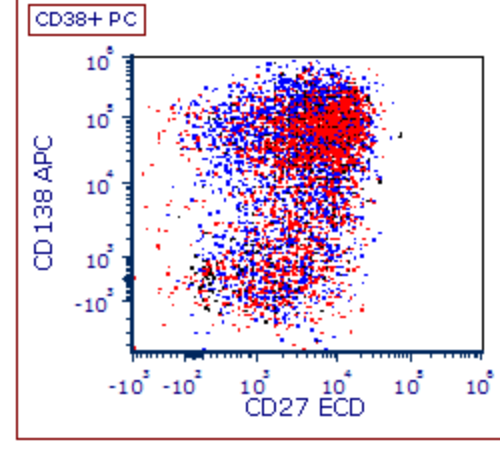
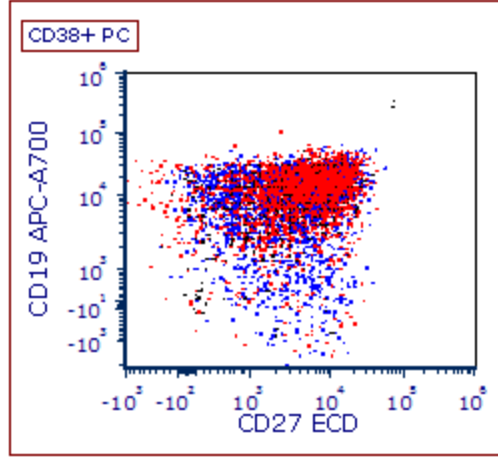
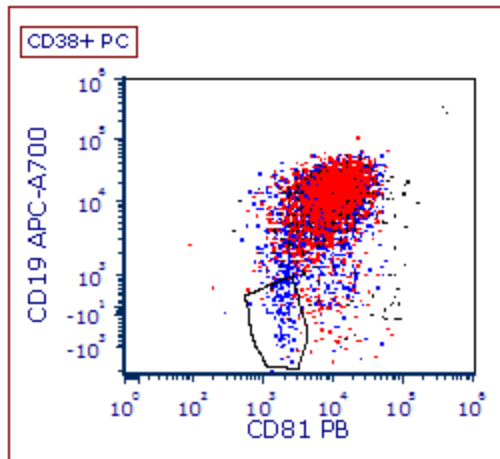
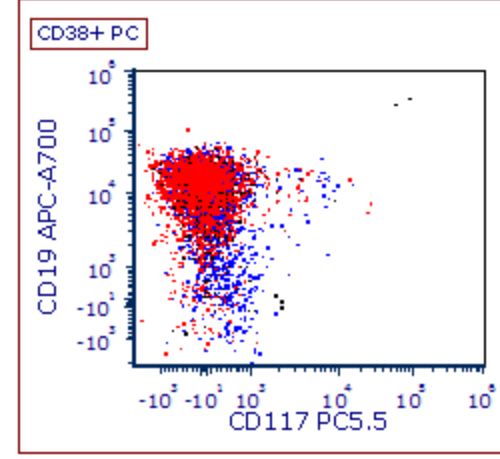
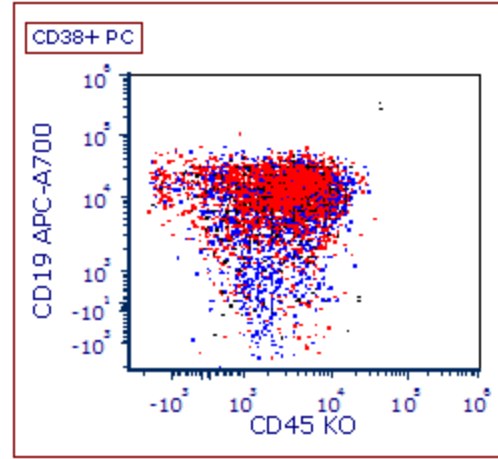
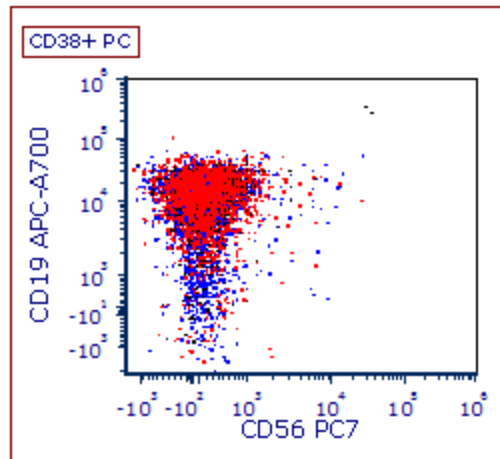
0.206% of leukocytes, 86% of PCs



0.0124%, 8% of all PCs



0.006%, 5% of all PCs



CLL MRD

- Similar story to MM MRD
- Decreased or increase expression of various markers
- CD43
- CD81
- CD20
- CD200
- CD22
- CD5

B-LBL and AML MRD

- Normal resident populations of hematogones and myeloid blasts are present
- Based on detection of abnormalities that take blast population outside of the regulated/coordinated expression of antigens

Important Clinical Questions

- Prognostic vs Predictive
 - » I know you'll do poorly but there is nothing I can do about it.
 - » I know you'll do poorly and I can treat you differently with good results.
- Distinction is based on clinical decision making
- Should payers support prognostic markers that are not predictive?
- Should clinicians use these markers?

Prognostic vs Predictive

- Predictive
 - » B-ALL – COG studies have showing early treatment in MRD+ cases lead to better results
 - » AML – some studies, SCCA, MDACC suggest early treatment in MRD+ at day 30 lead to better results
- Prognostic
 - » Myeloma MRD – no studies have show any treatment in MRD+ cases will lead to better outcomes
 - » CLL MRD - ?

Surrogate Endpoint

- Survivals for myeloma and CLL are measured in years
- Can MRD be used to predict poor outcomes of therapies? Very important for drug trials!
- FDA approval of CLL MRD as a surrogate endpoint



ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.