

New CAP requirements to improve MRD testing standardization

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Driven to DiscoverSM

Disclosures

- Vice-Chair, Diagnostic Immunology Resource Committee, College of American Pathologists
- Council Member, International Clinical Cytometry Society
- I receive royalties from Cell Signaling Technology, Danvers, MA, who uses technology that I created to produce monoclonal antibodies

College of American Pathologists

- Accreditation program – 7600 labs worldwide, through Checklists and peer inspections
- Proficiency testing/external quality assurance program – more than 20,000 labs worldwide subscribe to Surveys

Diagnostic Immunology Resource Committee

- Serve as CAP's scientific and educational resource for diagnostic immunology and flow cytometry
- Multiple “dry” and “wet” proficiency testing Surveys

Hypothetical clinical encounter

- A patient with a history of plasma cell myeloma undergoes bone marrow biopsy at institution A
- The patient has a close family member who works at reference lab B
- The bone marrow aspirate is split and sent to both labs A and B for MRD testing

Flow cytometry lab A

- Your patient's bone marrow biopsy is **POSITIVE** for minimal residual disease

Flow cytometry lab B

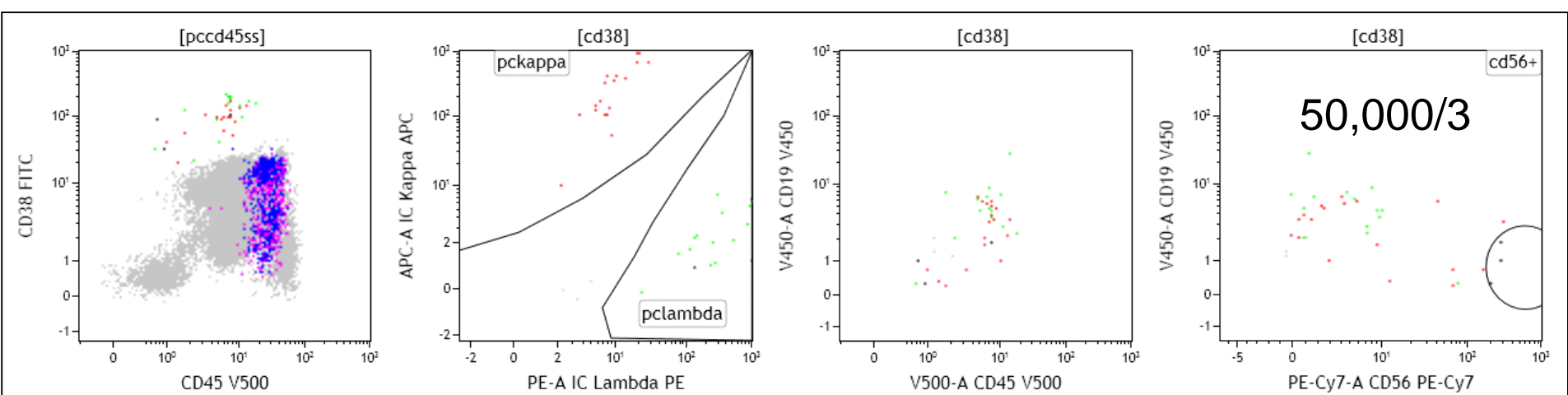
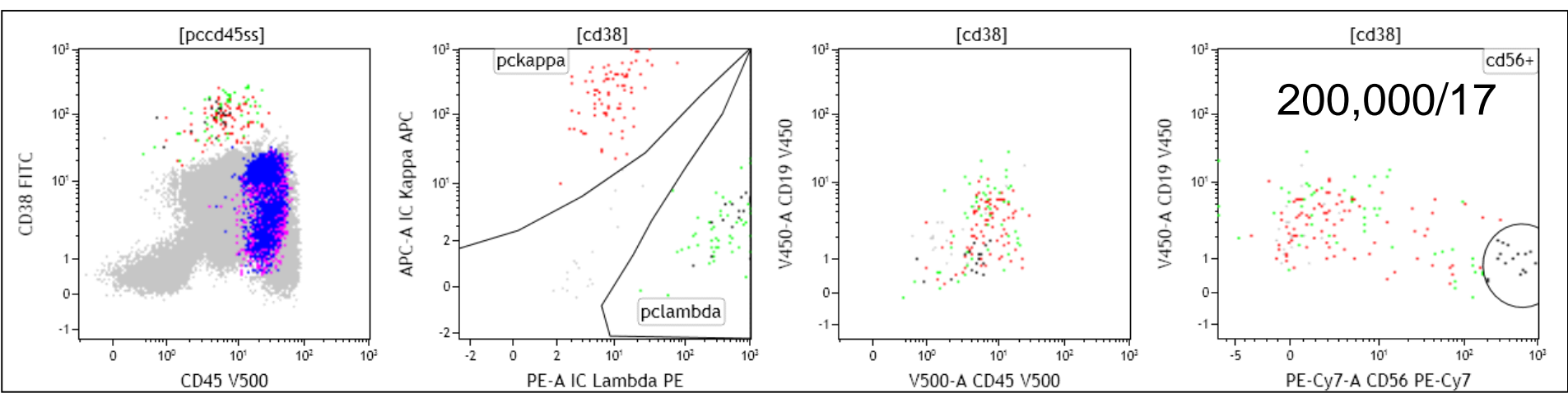
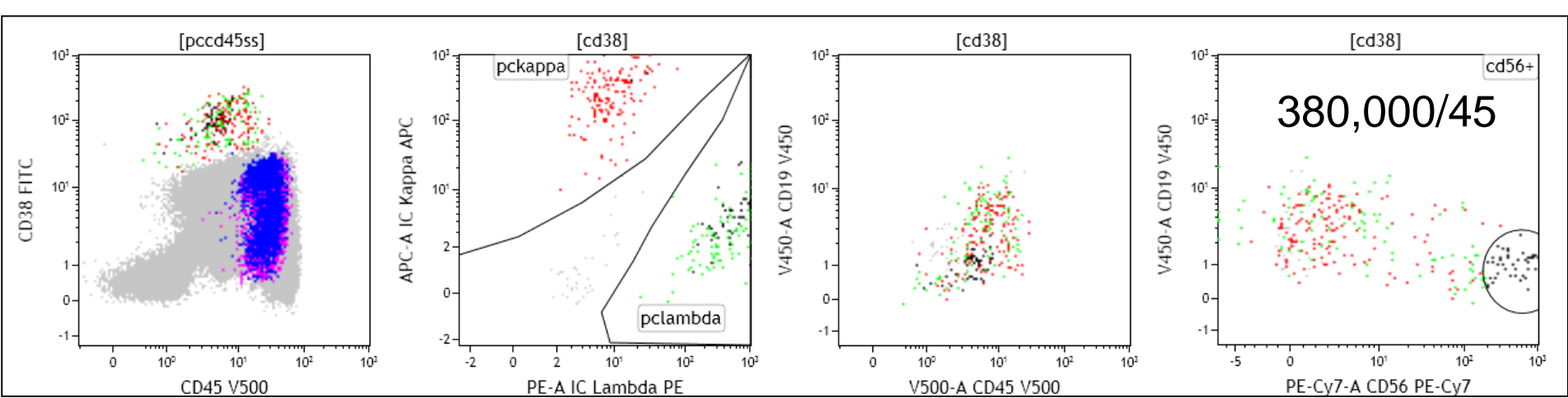
- Your patient's bone marrow biopsy is **NEGATIVE** for minimal residual disease

You call down to the lab and ask the pathologist....

- Why are the results discordant?
- What are the next steps?

Some factors affecting MRD limit of detection

- Patchy disease
- Aspirate hemodilution
- Staining method (including lysis)
- Number of “colors”/antigens studied
- Number of events collected



Major heterogeneity in PCM flow testing

Table 1. Flow cytometry of the bone marrow to detect MRD in multiple myeloma: current practice based on a survey of 30 major institutions in the United States

Institution	Number of events acquired in MRD testing	Minimum number of abnormal plasma cells needed to call MRD	Maximum possible sensitivity, %*	Number of antigens studied	Definition of MRD in bone marrow				
					CD38+, CD138+ with monoclonal light chains	CD38+, CD138+, CD19-, CD45-	CD38+, CD138+, CD19-, CD45-, CD56+	CD38+, CD138+ with dim or negative CD27	Other
1	3 000 000-4 000 000	20	0.0005	12	×			×	×
2	1 800 000	50	0.003	9	×				×
3	1 000 000	50	0.005	8		×	×	×	
4	500 000	25	0.005	6	×				×
5	500 000	NA		8	×				
6	300 000-500 000	30-35	0.006	8	×	×	×		
7	250 000-500 000	50	0.01	10	×	×	×	×	
8	100 000	20	0.02	10	×	×	×		×
9	100 000	Variable†	NA	8		×			×
10	Variable	20	NA	8		×	×		
11	NA	30	NA	8	×		×		
12-26	Do not perform MRD testing				Do not perform MRD testing				
27-30	Did not respond				Did not respond				

NA, not applicable.

*Maximum possible sensitivity determined by dividing the minimum number of abnormal plasma cells needed to call MRD by the number of events acquired in MRD testing (ie, a lower percentage indicates a more sensitive approach). For institutions with a range, the lowest number of minimum abnormal plasma cells needed and the highest number of events acquired were used for the calculation.

†Pathologist dependent.

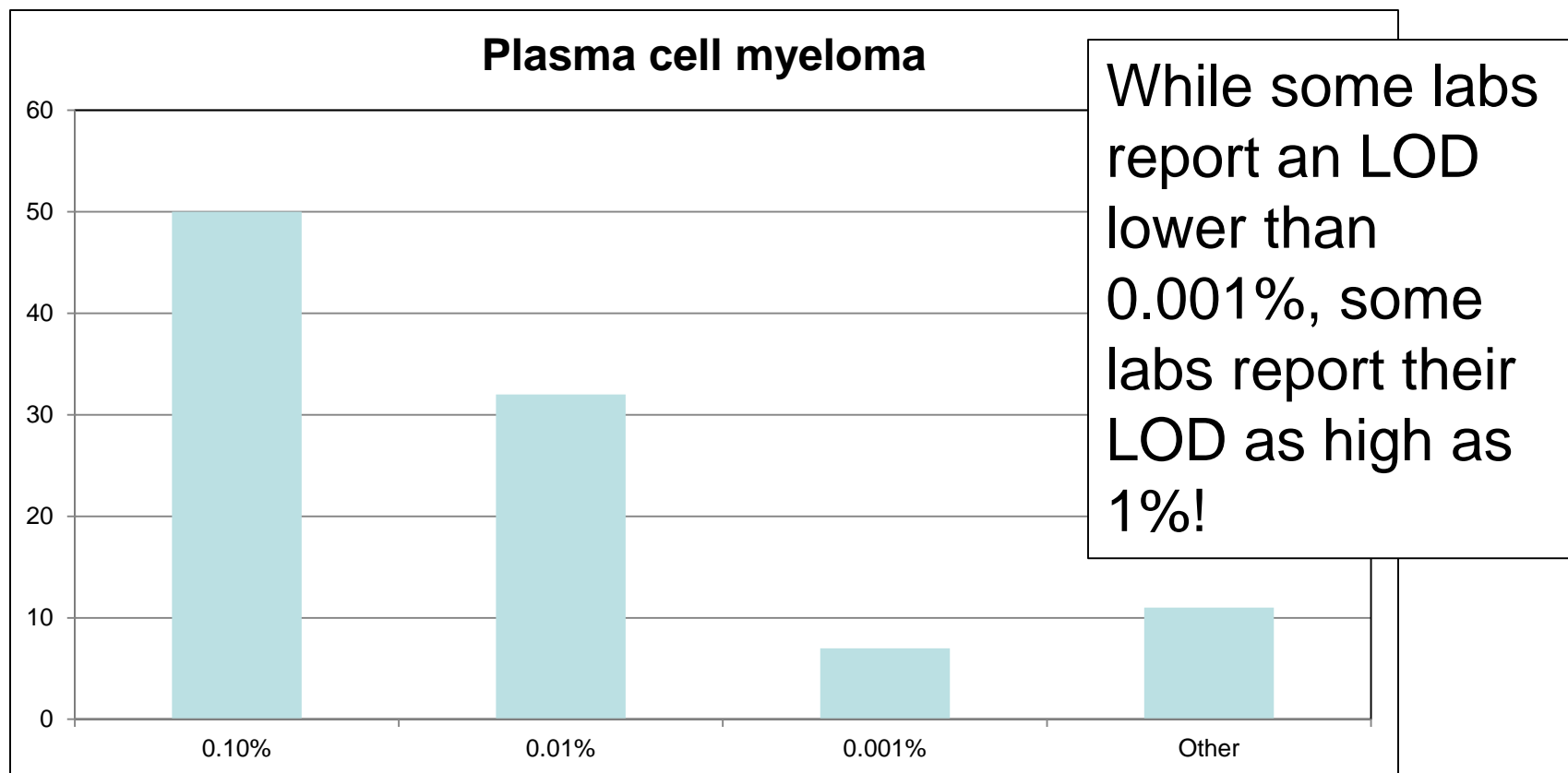
College of American Pathologists (CAP) MRD Survey

- Approximately 550 labs subscribe to our flow cytometry proficiency testing Survey (wet challenge)
- In 2014 we surveyed all labs to ask if they perform MRD analysis; we had 91% of labs complete the survey
- 91 labs (18% of respondents) perform MRD for myeloma
- These labs are worldwide, but predominantly in North America

Limit of detection (LOD)

- Survey was designed to ask about what the labs perceived (or measured) analytical LOD was for myeloma MRD
- We did not ask about number of events collected, number of “colors,” etc.
- Choices included 0.1%, 0.01%, 0.001%, and other

Reported LOD among 91 labs that perform MRD testing for myeloma



New changes for CAP Checklist

- Two new Checklist items created to specifically address rare event flow cytometric analysis/MRD
- Goal is to help clinicians and pathologists compare results from different labs and ultimately improve standardization

New Checklist Items

Rare Event Flow Cytometric Assays

****NEW**** 07/28/2015

FLO.30800 Rare Event Flow Cytometric Assays

Phase II

For rare event flow cytometric assays, the lower limit of enumeration has been validated.

NOTE: The detection of rare events may occur in assays, such as Paroxysmal Nocturnal Hemoglobinuria (PNH) clone testing or minimal residual disease (MRD) testing. Analytic sensitivity of the lower detection limit should be validated by performing dilutional studies using known patient or suitable reference material, such as proficiency testing material.

****NEW**** 07/28/2015

FLO.30820 Rare Event Flow Cytometric Assays

Phase I

For rare event flow cytometric assays, the lower limit of enumeration is included in the diagnostic report.

Example dilutional experiment

- Bone marrow sample has a plasma cell clone comprising 10% of leukocytes.
- 10 fold dilutions would be made into normal marrow so that the expected recovery would be 1%, 0.1%, 0.01%, 0.001% and 0.0001%
- All samples stained and run in parallel to find out limit of detection

Hypothetical scenario revisited

- A patient with a history of plasma cell myeloma undergoes bone marrow biopsy at institution A
- The patient has a close family member who works at reference lab B
- The bone marrow aspirate is split and sent to both labs A and B for MRD testing

Flow cytometry lab A

- Your patient's bone marrow biopsy is **POSITIVE** for minimal residual disease (*the measured and reported lower limit of detection for this lab's PCM MRD assay is 0.001%*)

Flow cytometry lab B

- Your patient's bone marrow biopsy is **NEGATIVE** for minimal residual disease (*the measured and reported lower limit of detection for this lab's PCM MRD assay is 0.01%*)

Questions now answered by the provider

- Why are the results discordant? *Lab A has a more sensitive method than Lab B*
- What are the next steps? *Varies, but the main thing to remember is that:*
 - *Lab A \neq Lab B*

Summary

- There is major heterogeneity in how the labs surveyed define MRD for PCM by flow cytometry
- Leukemia/lymphoma and MRD testing by flow cytometry are laboratory developed tests
- National and international professional organizations, as well as market pressures, will continue to encourage the field to standardize/improve

QUESTIONS?
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