Biomarker assays

Fit-for-purpose validation

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Context of use of Biomarkers analysis

→ Biomarker for clinical diagnosis of patients: to include one disease and exclude the other
→ Biomarker to judge the effect of a therapeutic

The same assay may be used for both contexts
The context of use (CoU) is often so different that this requires additional validation

To change CoU for an assay:

→ Pre-validation experiments will give context to the required (additional) validation of the test

→ Fit-for-purpose additions will complete existing validations for routine analysis

From routine diagnostics towards biomarker testing for (pre-)clinical trials

- ISO15189 validation
- Biomarker fit-for-purpose validation for sponsors

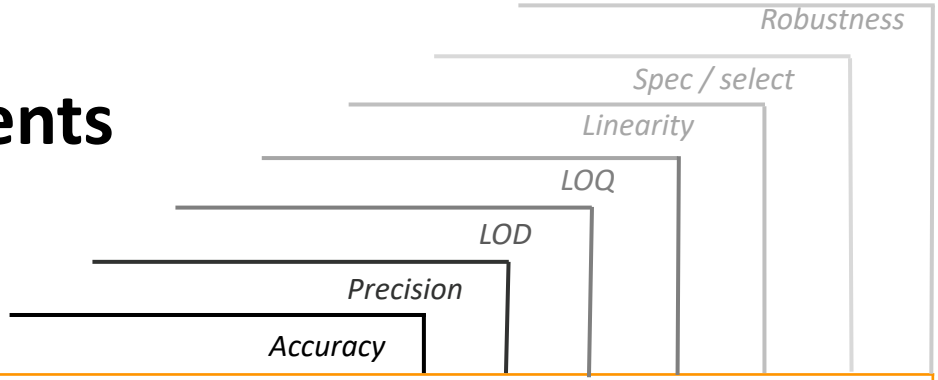
→ Translation between the two

How to validate in a fit-for-purpose fashion?

Two examples

- anti-MOG flowcytometry assay
- complement activation markers

Routine diagnostics validation requirements



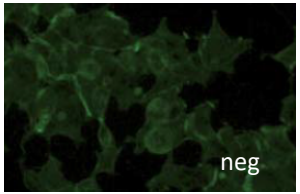
Type of method								
New	Qualitative	o	o	•	o	o	•	•
	Quantitative (high conc)	•	•	o	o	•	•	•
	Quantitative (low conc)	•	•	•	•	o	•	•
Standard	Qualitative	o	o	•	o	o	o	o
	Quantitative (high conc)	•	•	o	o	o	o	o
	Quantitative (low conc)	•	•	•	o	o	o	o
Adapted	Qualitative	o	o	•	o	o	o	o
	Quantitative (high conc)	•	•	o	o	o	o	o
	Quantitative (low conc)	•	•	•	•	o	o	o

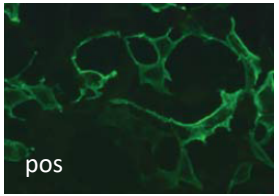
• required
o not required

Anti-MOG antibodies CBA validation

MOG = myelin oligodendrocyte glycoprotein
Marker for CNS demyelination (non-MS, anti-AQP4 neg)

Cell-based assay is gold standard: semi-quantitative, specific, sensitive
IFT is less sensitive and qualitative





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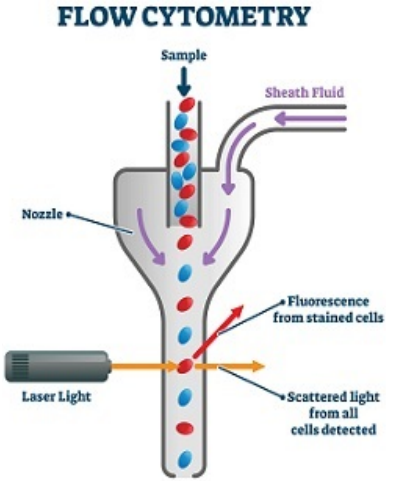


www.labcompare.com

AIM

To validate the assay.

However: quantitative result and visual judgement of dotplots did not correlate well.

To have a fit-for-purpose analysis strategy in order to diagnose patients correctly.
Determine clinical value of the cut-off used: first look into analysis strategy

Assay principle

Transduced cell
MOG + GFP

Patient antibody

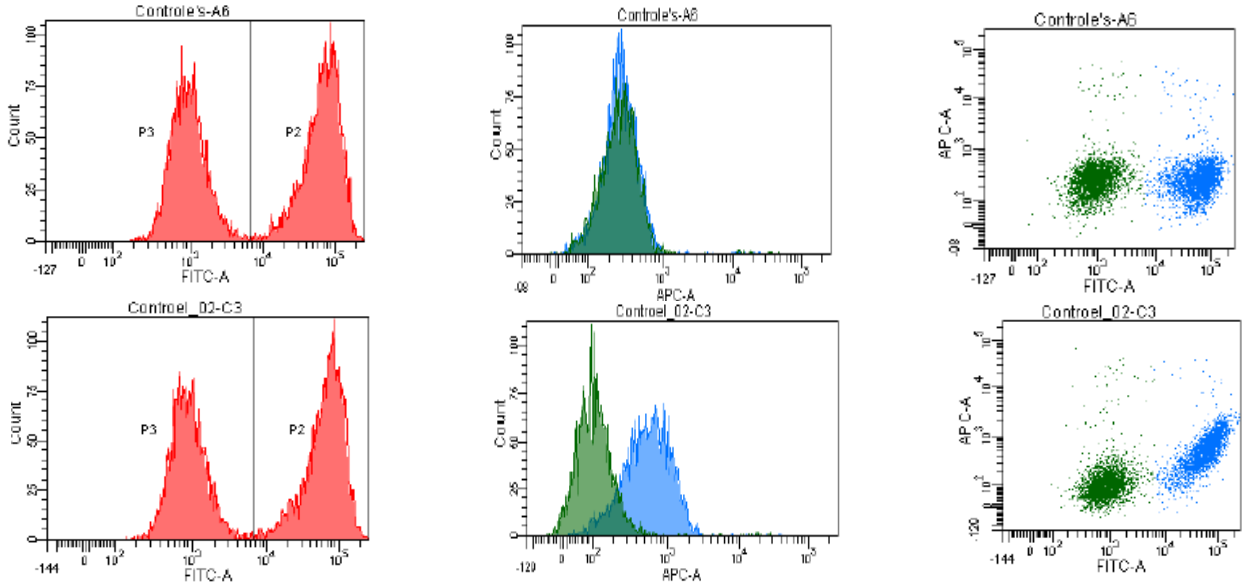
Anti-hIgG-APC

Untransduced cell

Cells are mixed during analysis

Negative sample

Positive sample



Mean fluorescence intensity (MFI)

Cut-off determination

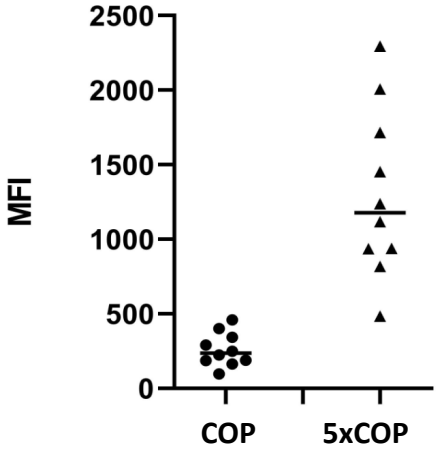
The result is expressed as ΔMFI (MFI transduced cells – MFI untransduced cells)

- Cut-off for weakly pos (COP) is set at the average ΔMFI + **10SD** of 8 negative controls
- Cut-off for positive is 5xCOP

Neg < (mean + 10SD)

(Mean + 10SD) < Weakly pos < 5x (mean+10SD)

Pos > 5x (Mean +10SD)



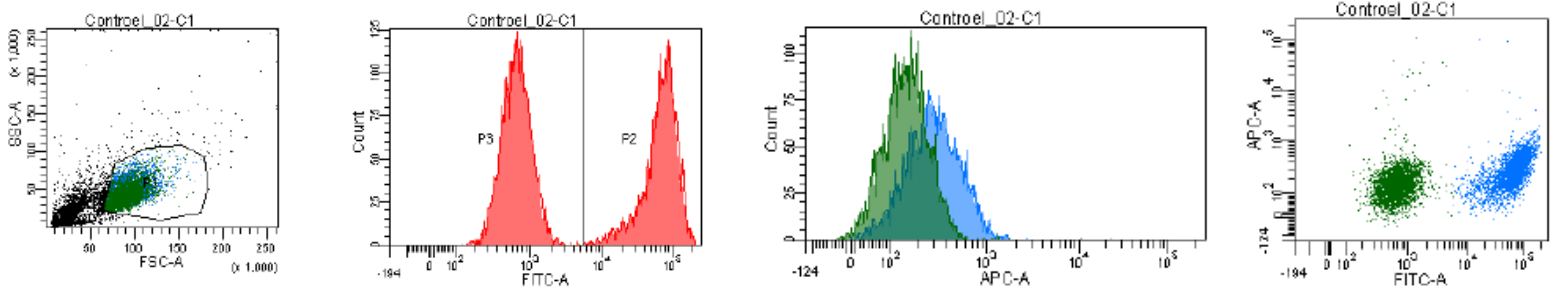
Aim

Prevalidation: retrospective analysis for several aspects of flow-analysis

Prevalidation: visual analysis

SOP describes to look both at dotplot and the **ΔMFI**

COP in this run: 249
ΔMFI sample: 126



Discrepancies between visual calulated result	
Number of samples analysed	272
Number of discrepancies visual vs numbers	30 (11%) (N → WP)

→ To look into the calculation

Prevalidation: cut-off determination

Tea et al. (2020) suggested that a COP of mean +6SD or mean+3SD would give a better sensitivity.

BUT: Prevent false positives!

Discrepancies when using +10SD versus +6SD versus +3SD	
Number of samples analysed	272
Number of discrepancies +10SD vs +6SD	6 (2 N→WP, 4 WP→P)
Number of discrepancies +10SD vs +3SD	13 (4 N→WP, 9 WP→P)
Number of discrepancies +6SD vs +3SD	7 (2 N→WP, 5 WP→P)

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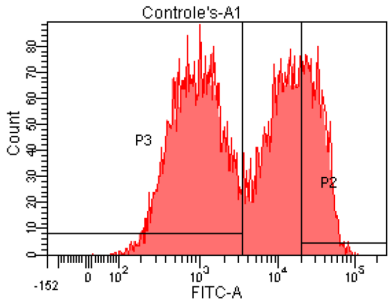
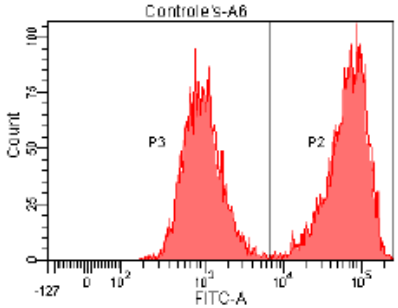
Discrepancies between visual calulated result	
Number of discrepancies visual vs +10SD	30
Number of discrepancies visual vs +6SD	27
Number of discrepancies visual vs +3SD*	25

* Two samples were positive based on numbers, but negative visually (false positives?)

Prevalidation: gating strategy

Previous gating strategy



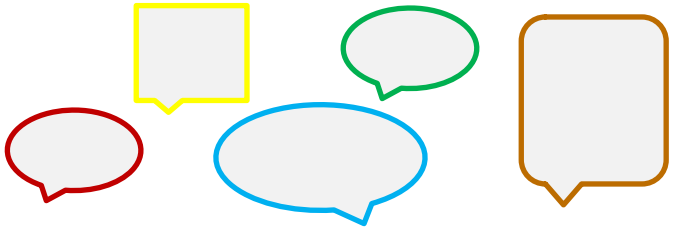


Pre-validation gating strategy

Discrepancies when gating on GFPhigh cells vs on whole GFP peak	
Number of samples analysed	272
Number of discrepancies	6 (all from neg to WP)
% of discrepancies	2.2%

Conclusion: gate whole peak, use 6SD instead of 10SD, visual interpretation?

Discussion with stakeholders



The adapted analysis methods make the assay more sensitive.
Check if this change in analysis fits the clinic?

→ Too many weak positives that clinically had MS-like disease

Share a lists of patient properties (clinical picture, lab results, imaging results),
Discuss these with the clinicians

→ Decide together if these changes in analysis would improve the diagnostic process

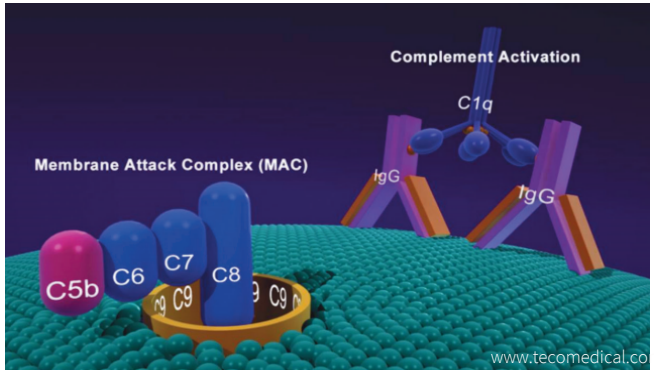
Than: start validation according with this set-up.
(LOD, Precision, linearity, clinical spec/sens)

Biomarkers to analyse complement activation or inhibition

What to measure:

- Remaining ability to be activated
- Concentration of proteins
- Activation markers
- Induction of autoantibodies

In routine: proof of complement *activation*
In studies: proof of complement *inhibition*



<u>Different needs between routine and trials</u>:

In routine diagnostics an increase in activation marker is indicative for a disease with complement activation

Samples will be measured within two weeks and after that dispersed off

When clinically inhibiting complement, a decrease is expected

Samples are collected and stored for longer times

Fit-for-purpose addition to the initial ISO15189 validation

Long term stability (LTS): multiple options

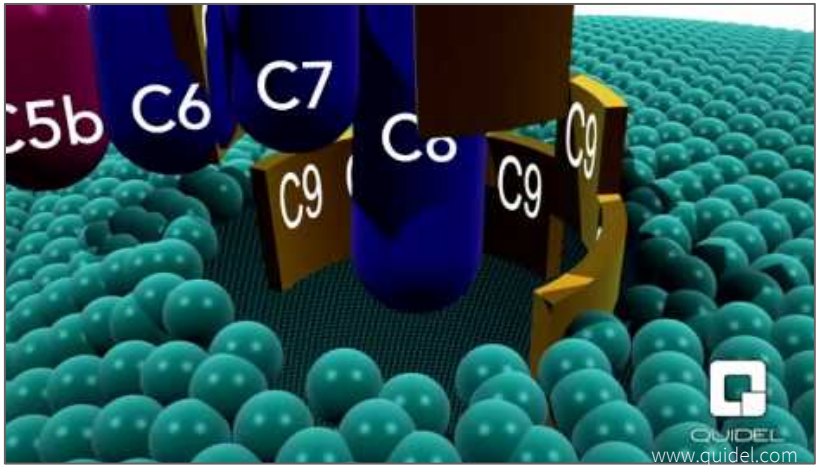
- Use control values retrospectively
- Remeasure old samples that have been stored (because the might be needed for assay improvement)
- Start LTS with new sample:

How to obtain a positive sample?

- From routine with informed consent
- Spiking (but often difficult eg sC5b-9)
- Activate serum at 37°C and add EDTA

Determine LOQ

- Extrapolate under lowest standard point?
- Linearity at LOQ/MRD



In summary

- The CoU determines the set-up of the assay
- Discuss with the sponsors to determined the CoU & fit-for-purposeness
- With prevalidation experiments, the conditions to achieve the CoU were determined
- Retrospective analyses suggested the assay is fit-for-purpose with adapted cut-off
- Validation will follow, after detemining the conditions to achieve CoU

Thank you

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