SIEMENS Healthineers

N Latex FLC kappa and lambda assays

Customer roundtable meeting, Belgium

Dr. Christian Mirwaldt, Global Marketing
Sep. 2022

Overview

SIEMENS Healthineers

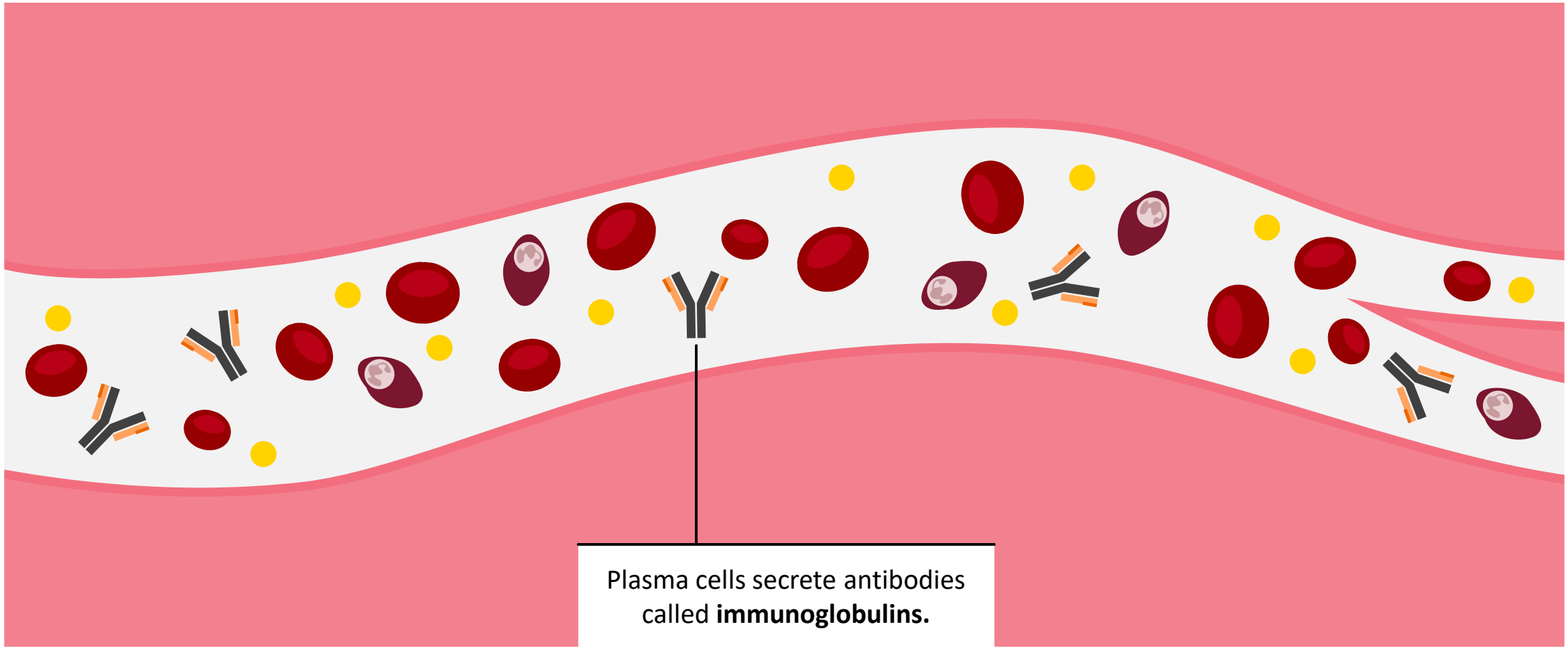
Dr. Henry Bence Jones

Monoclonal free light chains in urine of myeloma patient

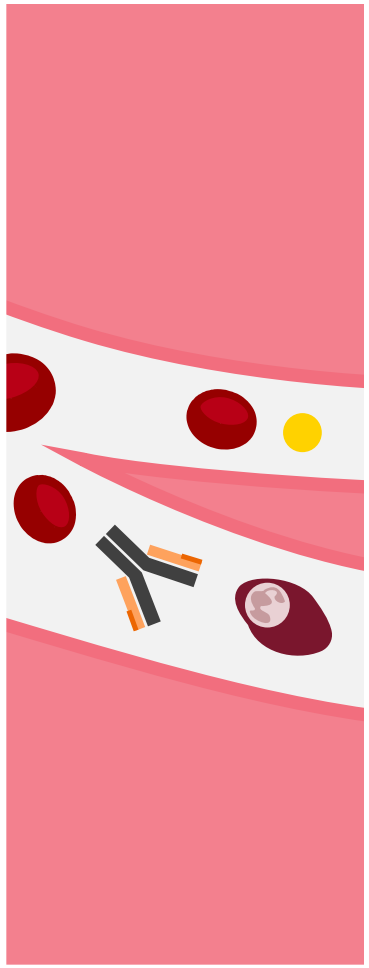
"The tube contains urine of very high specific gravity. When boiled it becomes highly opaque. On the addition of nitric acid, it effervesces, assumes a reddish hue, and becomes quite clear; but as it cools assumes the consistence and appearance which you see. Heat reliquefies it."

1847 description of Bence Jones proteinurea by Dr. Henry Bence Jones (1813-1873): http://en.wikipedia.org/wiki/Henry_Bence_Jones.

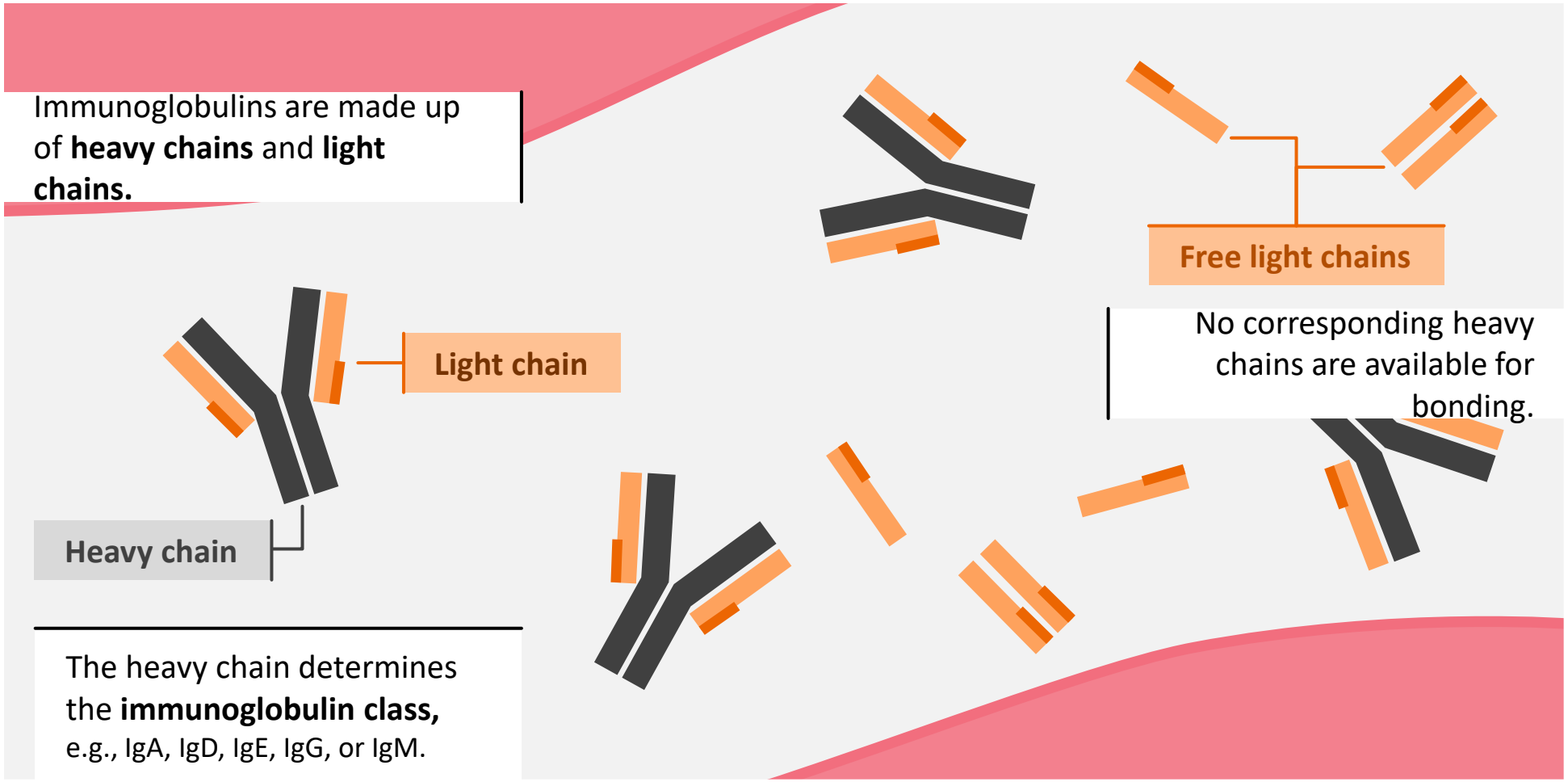
Immunoglobulins and free light chains



Immunoglobulins and free light chains



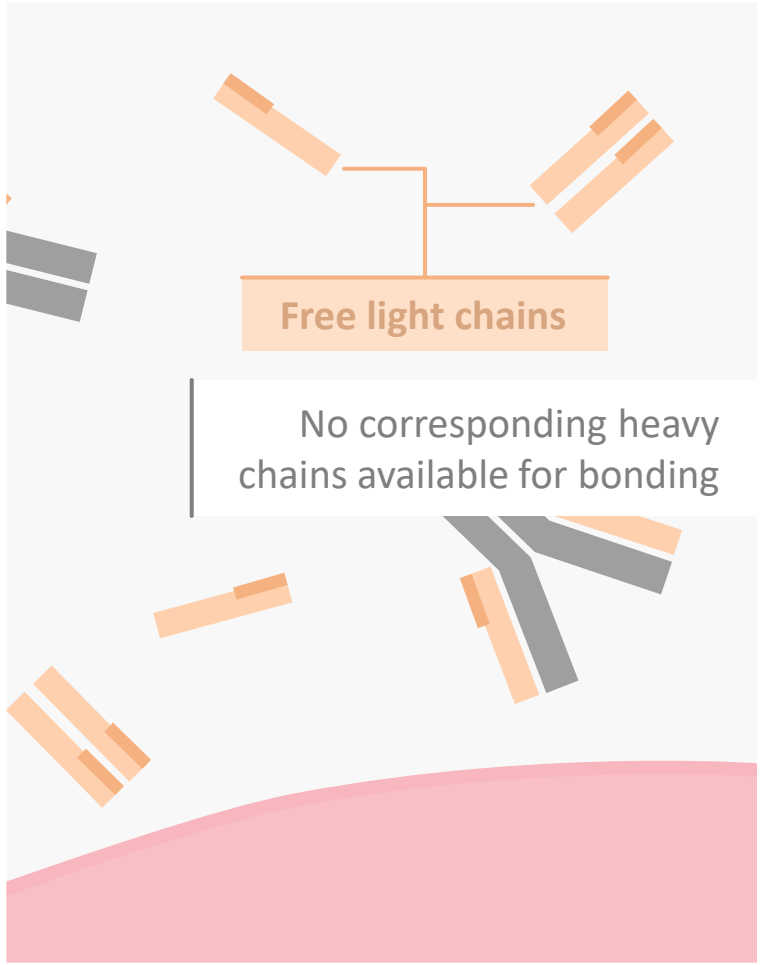
Immunoglobulins are made up of **heavy chains** and **light chains.**



No corresponding heavy chains are available for bonding.

The heavy chain determines the **immunoglobulin class,** e.g., IgA, IgD, IgE, IgG, or IgM.

Free light chains: kappa and lambda



Free **kappa** light chains

Mostly detectable as **monomers**

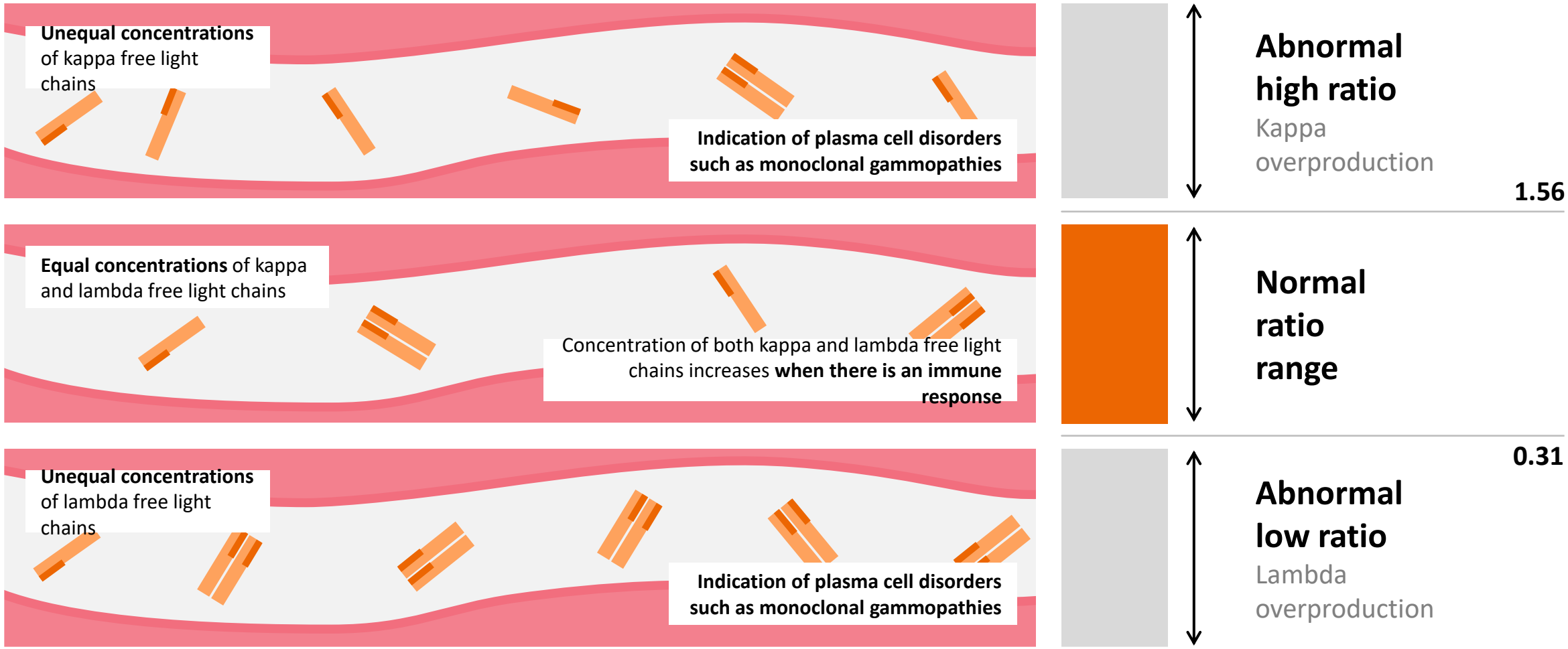
Half-life time: **2–3 hours**

Free **lambda** light chains

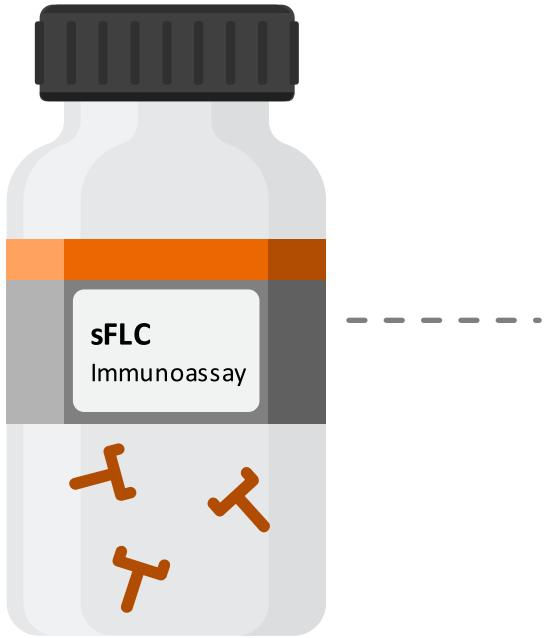
Mostly detectable as **dimers**

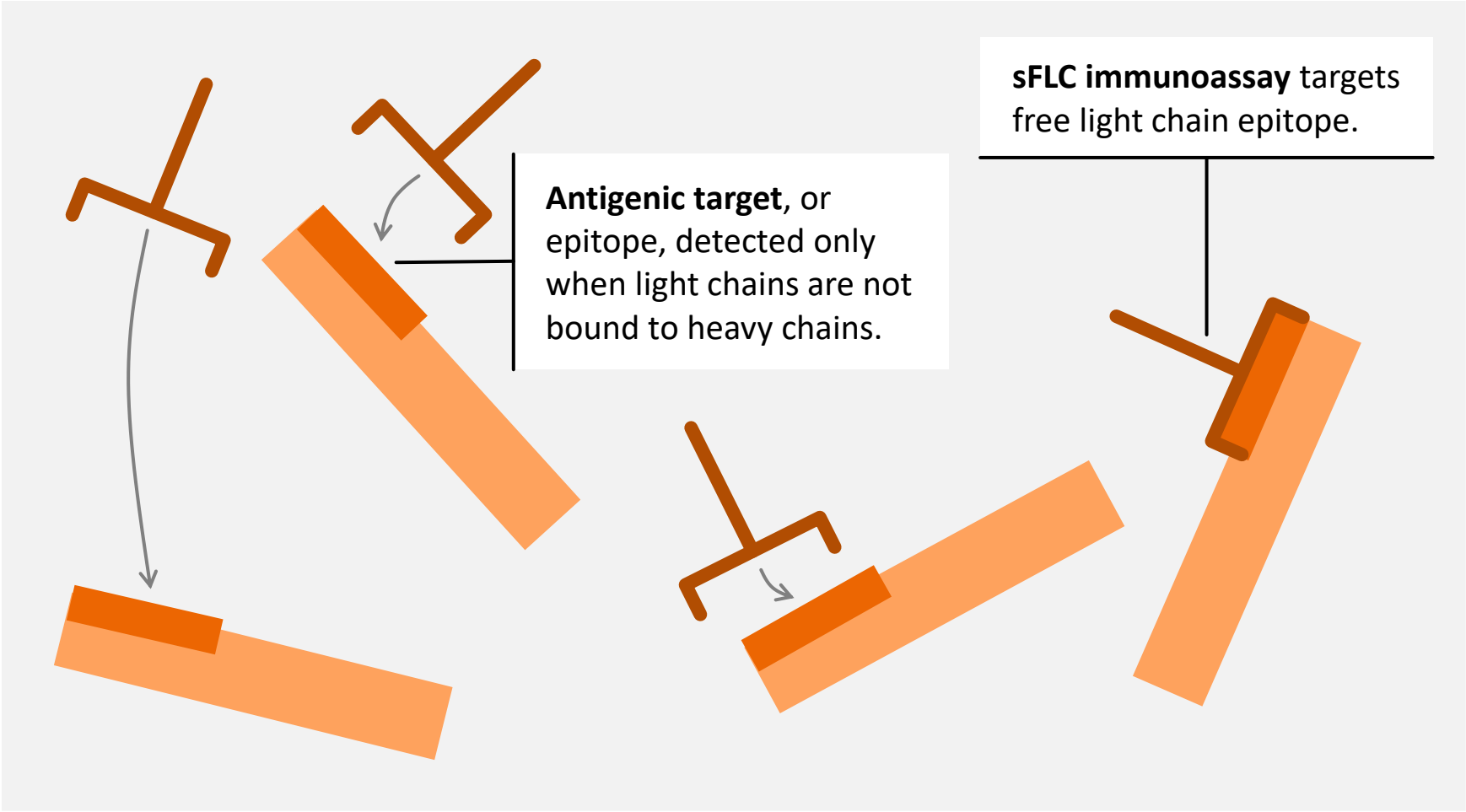
Half-life time: **4–6 hours**

Free light chain ratios



Antigenic targets



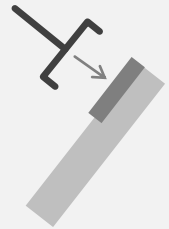


Free light chain: assay requirements

Latex assays based on **monoclonal antibodies**

Antigen-excess security
due to pre-reaction protocol

Flexible kit concept

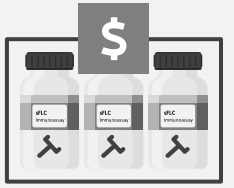


Accurate

Highly selective

High lot-to-lot consistency

Wide measuring range



Economical use of assay components

Overview

SIEMENS Healthineers

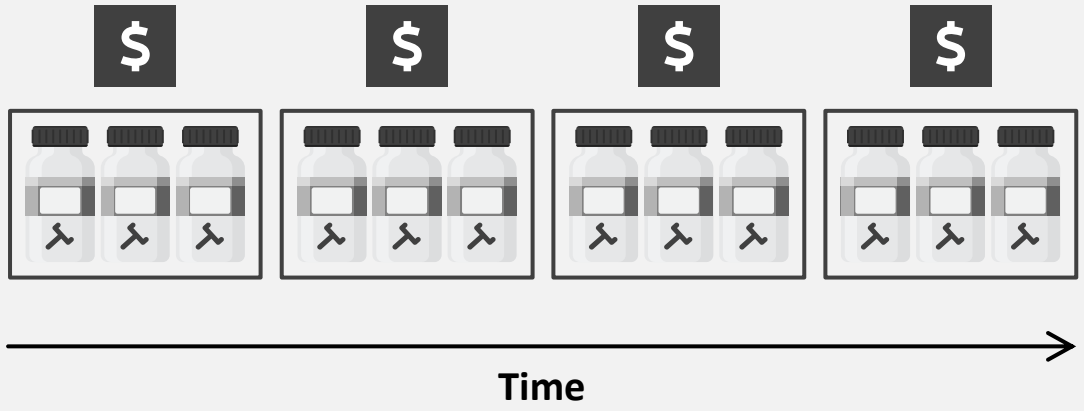
Kit configurations and components

Flexibility and value for money

SIEMENS Healthineers

FREELITE Assays

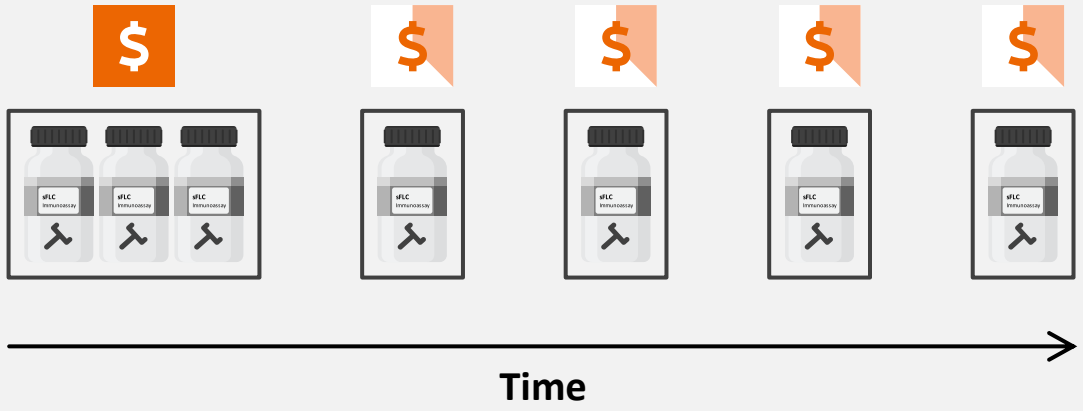
Order a new kit each time a component runs out.



Unnecessary waste and **potential higher cost**

N Latex FLC Assays

Order only the components you need.

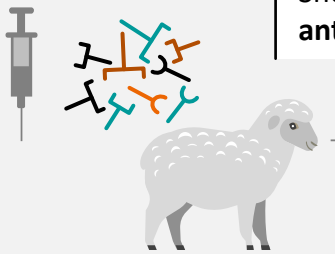


- Components can be used **interchangeably** and **lot-independently**
- **Minimized waste** and **reduced cost**

Monoclonal antibodies: why?

Antibody production process: polyclonal vs. monoclonal

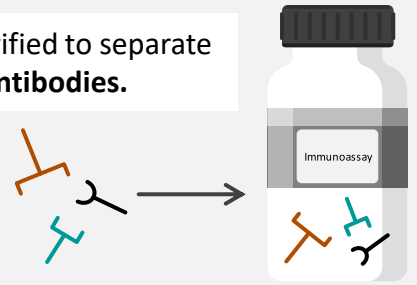
Immunization



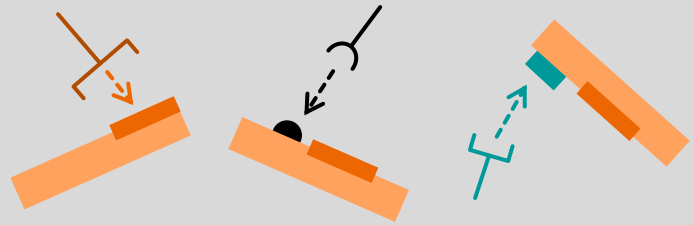
Sheep produces **antibodies.**

Blood taken from sheep.

Blood purified to separate **desired antibodies.**



>100 different antibodies that react against a specific antigen, **each identifying a different epitope.**



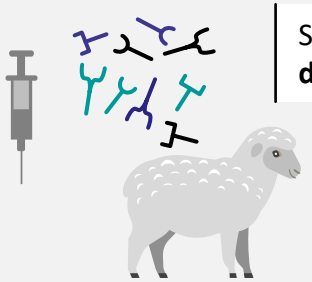
Patients with **myeloma**

! Need to **re-immunize** with every lot produced.

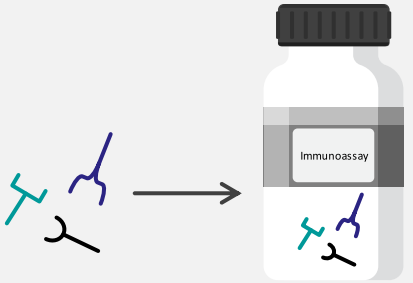
! **Different antigen pools** cause variation between lots.

! **Variation** in detection between lots.

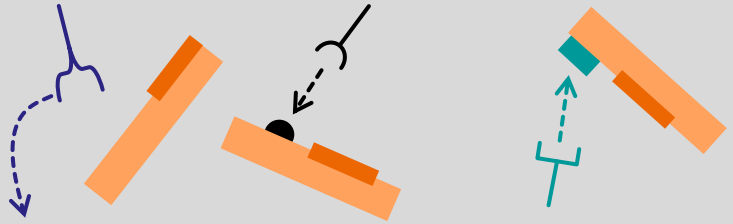
Re-immunization



Same sheep produces **different antibodies.**

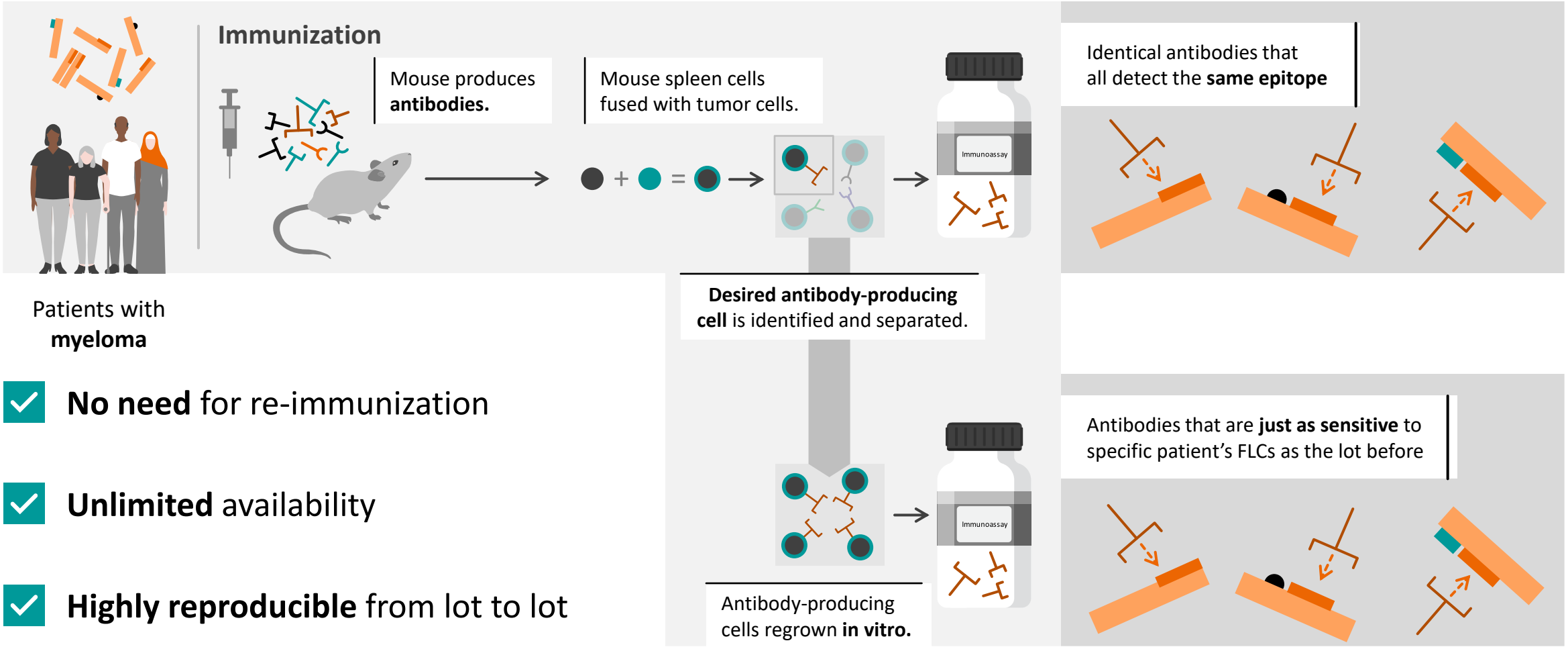


Antibodies may be **less-sensitive** to specific patient's FLCs than the lot before.



Monoclonal antibodies: why?

Antibody production process: polyclonal vs. monoclonal



- ☑ **No need** for re-immunization
- ☑ **Unlimited** availability
- ☑ **Highly reproducible** from lot to lot

Overview

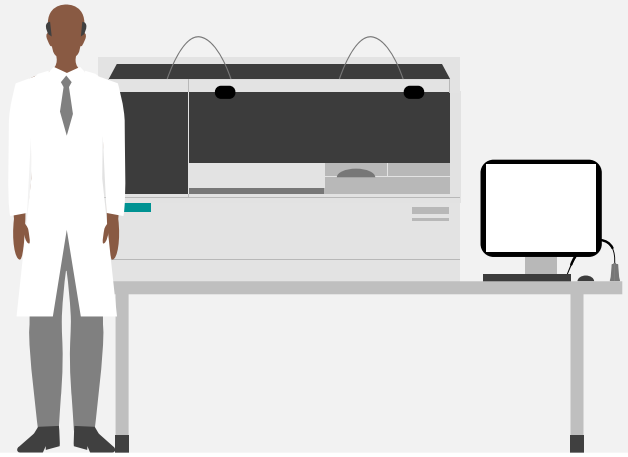
SIEMENS Healthineers

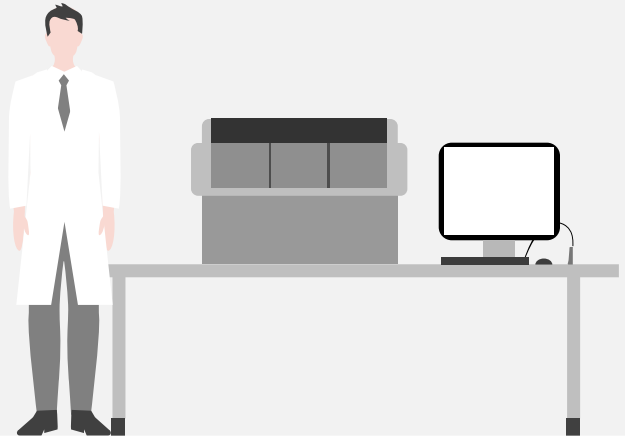
N Latex FLC assays: equivalent result quality

FLC clinical sensitivity: comparison to immunofixation

N Latex FLC Assays		Immunofixation		FREELITE Assays	
60	**58**	**60**	**59**	**59**	**56**
Kappa positive samples	**Lambda** positive samples	**Kappa** positive samples	**Lambda** positive samples	**Kappa** positive samples	**Lambda** positive samples

N Latex FLC assays are at least as sensitive as FREELITE assays.



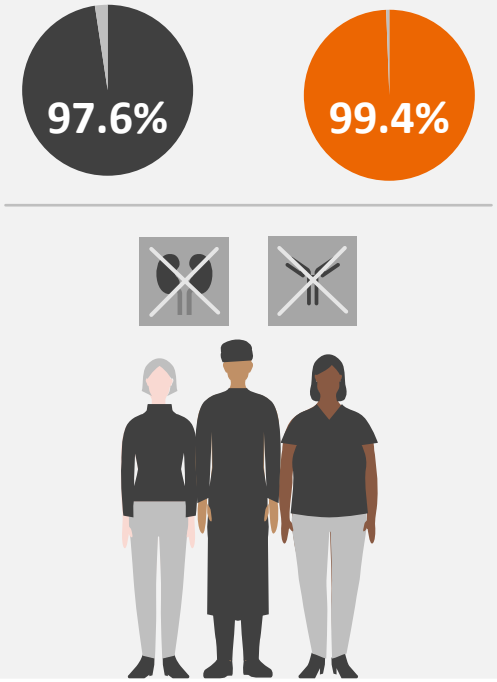


Data obtained during validation at Jeroen Bosch Hospital, Den Bosch, NL.

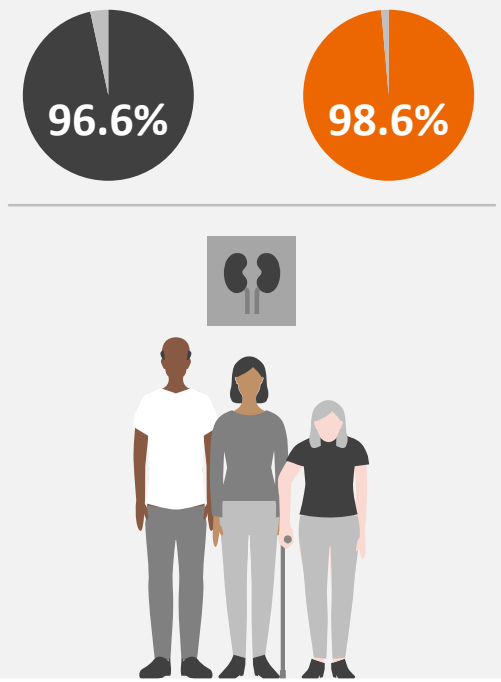
N Latex FLC assays: equivalent result quality

High clinical specificity

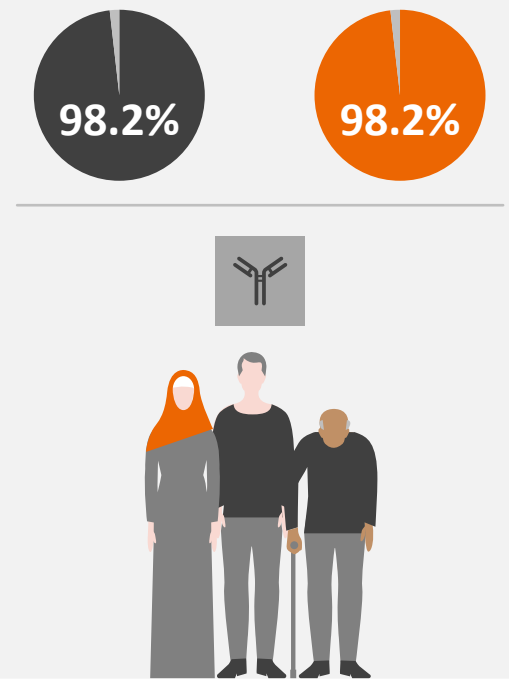
In patients without renal disease or polyclonal stimulation



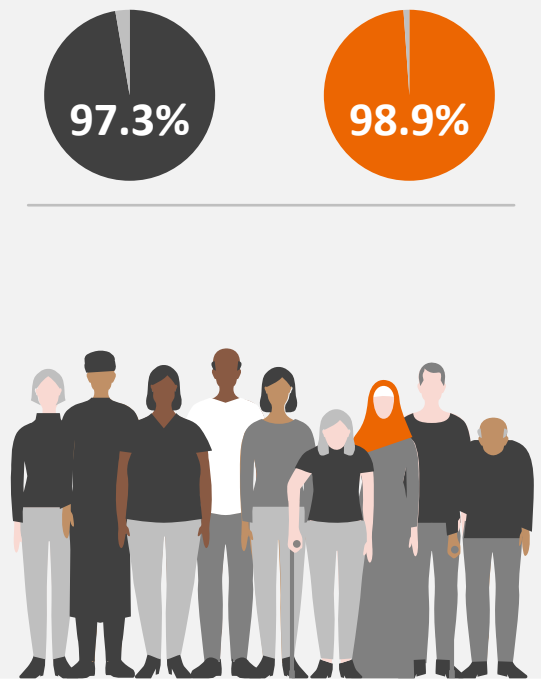
In patients with renal disease



In patients with polyclonal stimulation



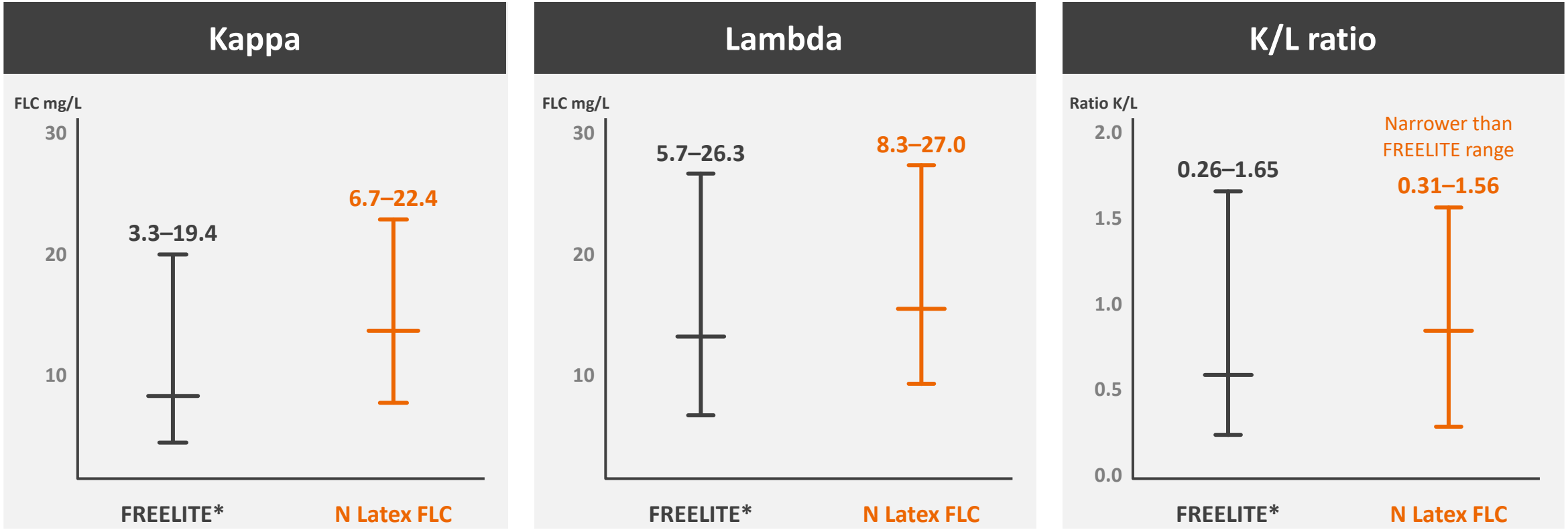
Overall specificity



Data obtained during validation at Jeroen Bosch Hospital, Den Bosch, NL.

N Latex FLC assays: comparable reference ranges

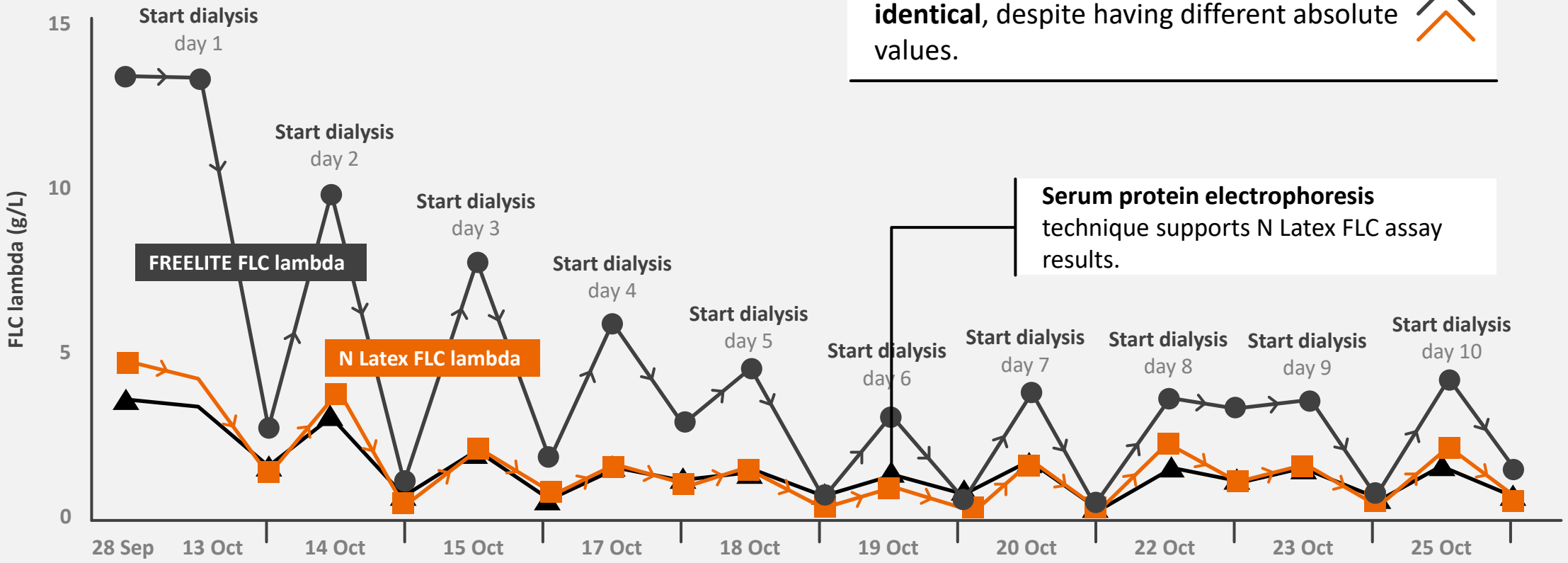
The majority of FREELITE and N Latex assay readings are contained within a similar range.



*FREELITE reference ranges from Optilite Freelite package insert: NS016.OPT , 05-2017 values established on BN™ II System)

N Latex FLC assays: similar detection behavior

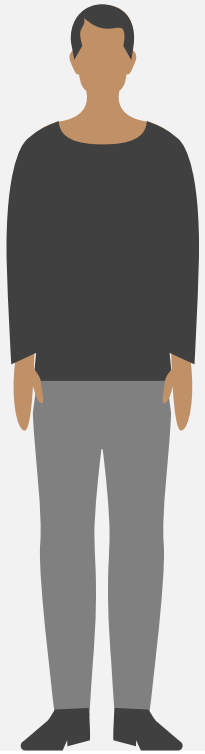
Patient with light chain lambda myeloma on dialysis with Campro filter

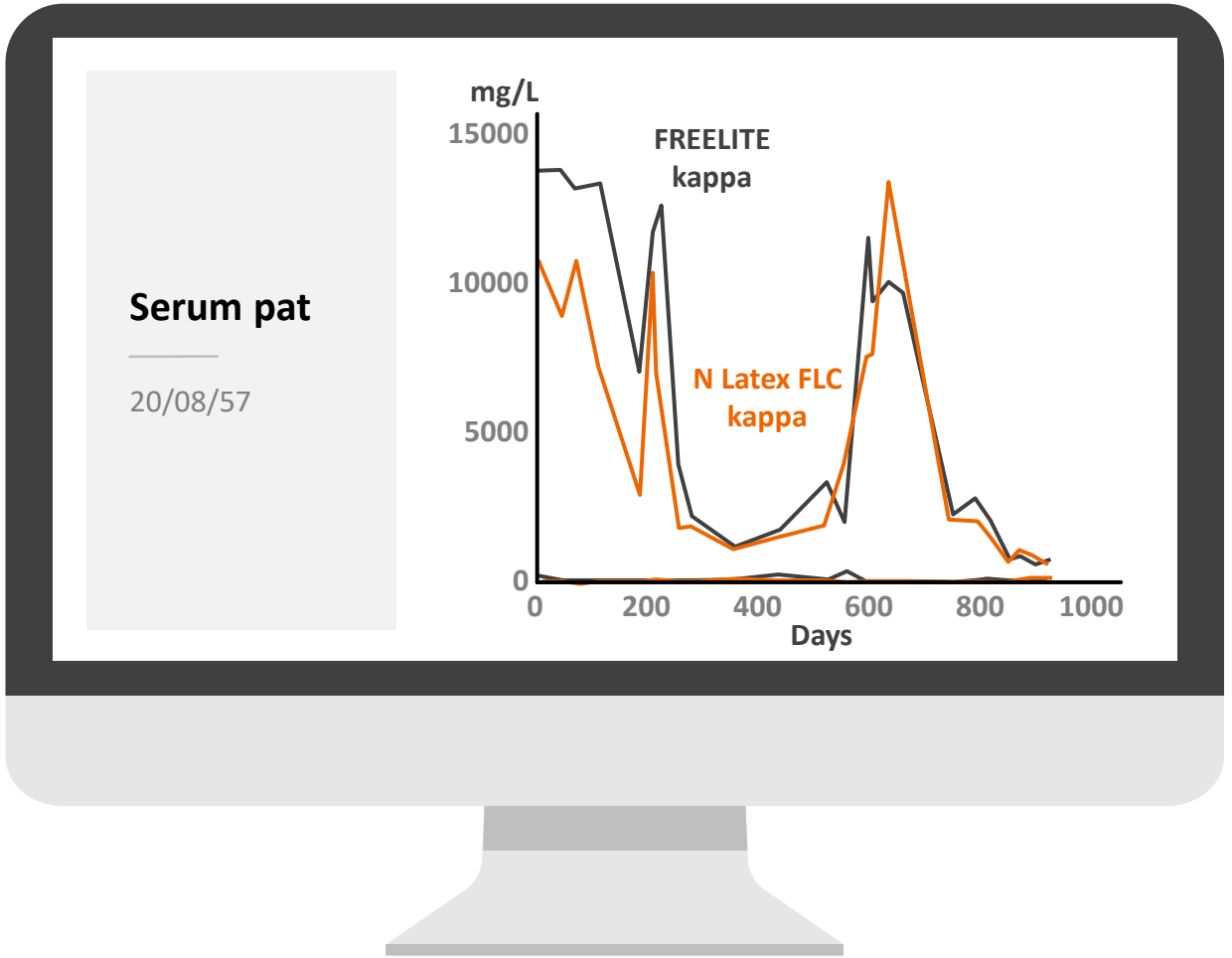


Gentzer W. Poster. AACC 2011.

Patient parallel testing: FLC kappa

Patient tested using **FREELITE** and **N Latex FLC** assays.





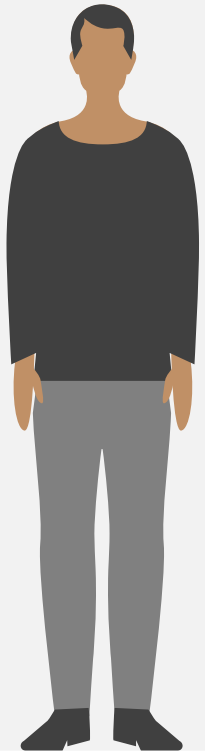
In a lot of cases Siemens Healthineers assays represent patient's situation better.

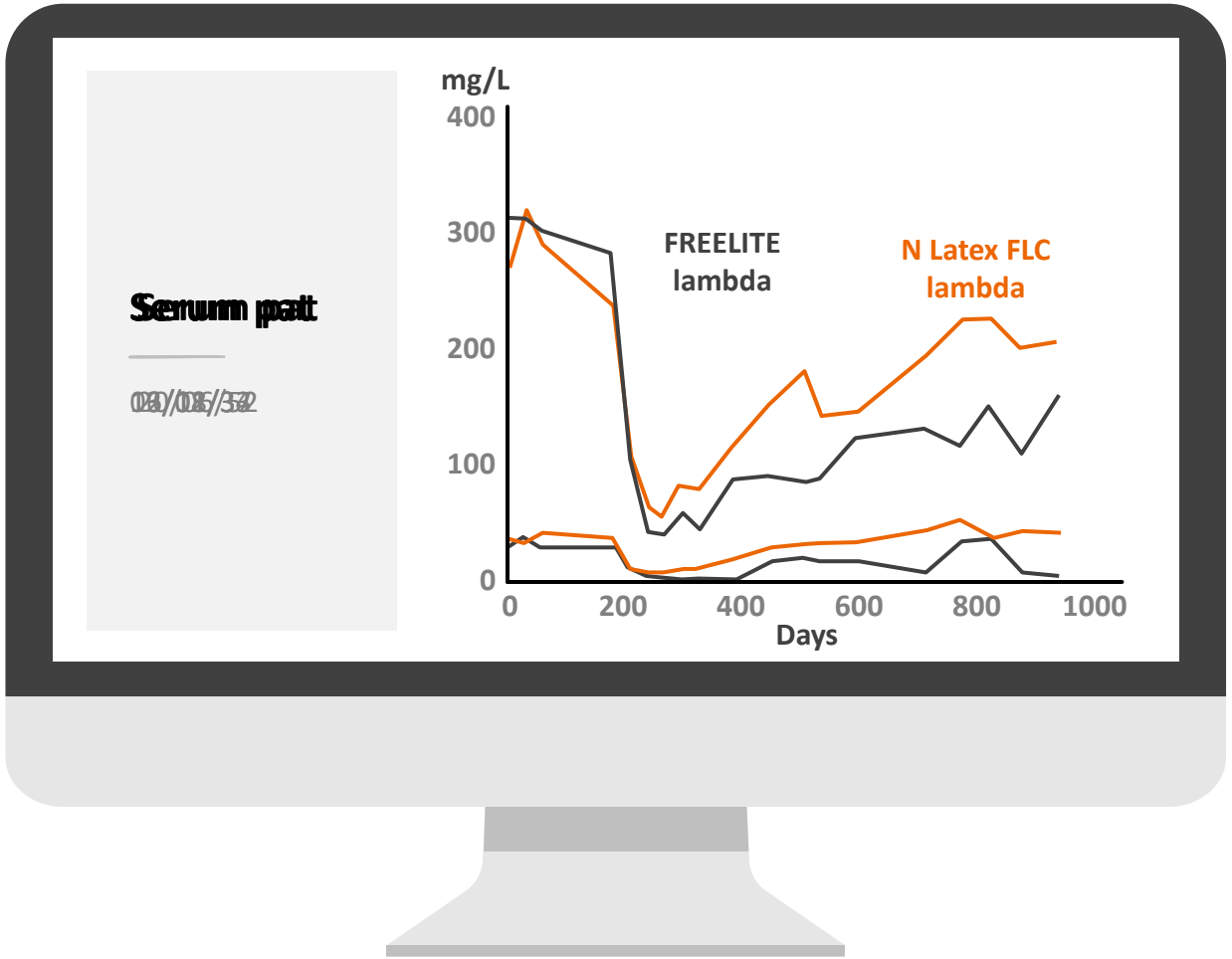
Reduced risk of misinterpretations.

Data obtained during validation at Jeroen Bosch Hospital, Den Bosch, NL.

Patient parallel testing: FLC lambda

Patient tested using **FREELITE** and **N Latex FLC** assays.



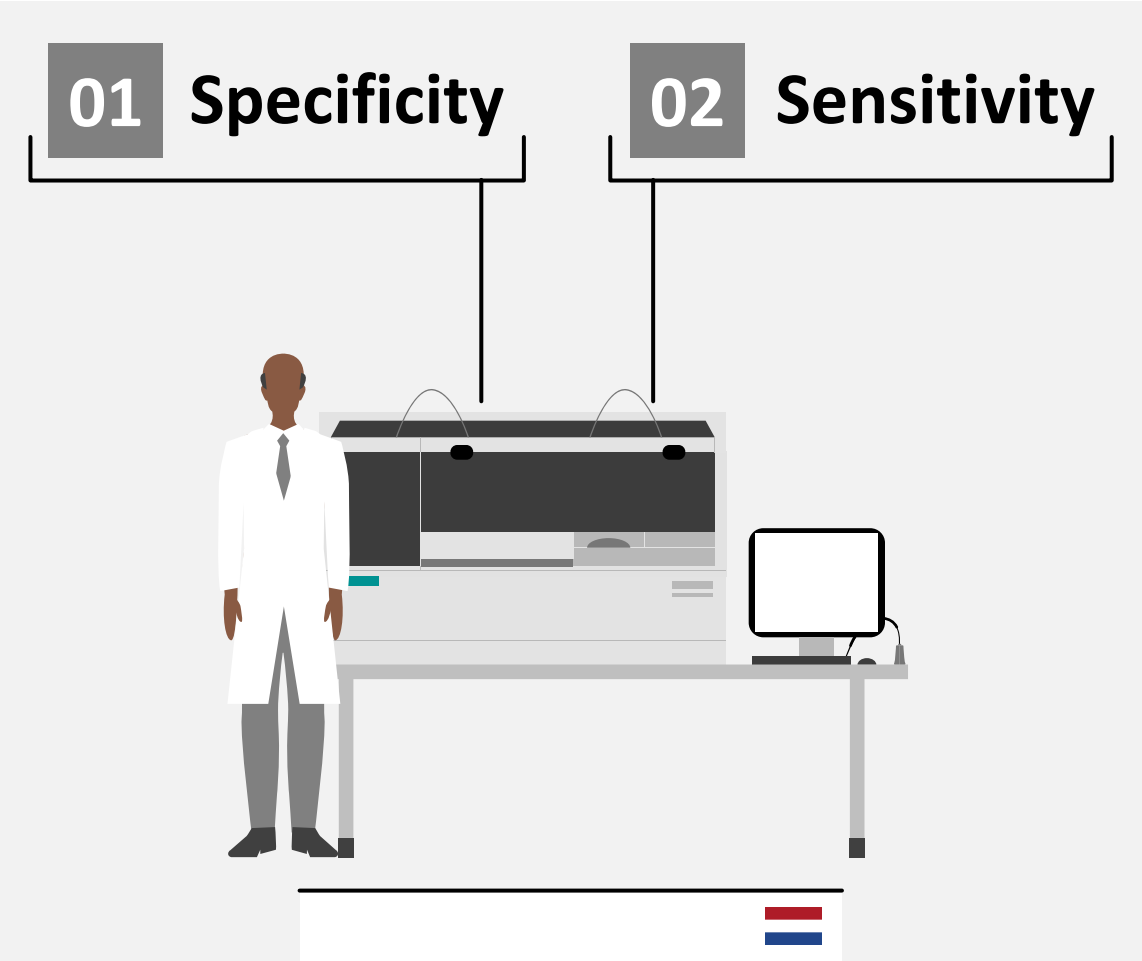


In a lot of cases also N Latex Lambda assay has high values, like FREELITE assay.

Data obtained during validation at Jeroen Bosch Hospital, Den Bosch, NL.

Clinical validation

Jeroen Bosch Hospital, Den Bosch, Netherlands

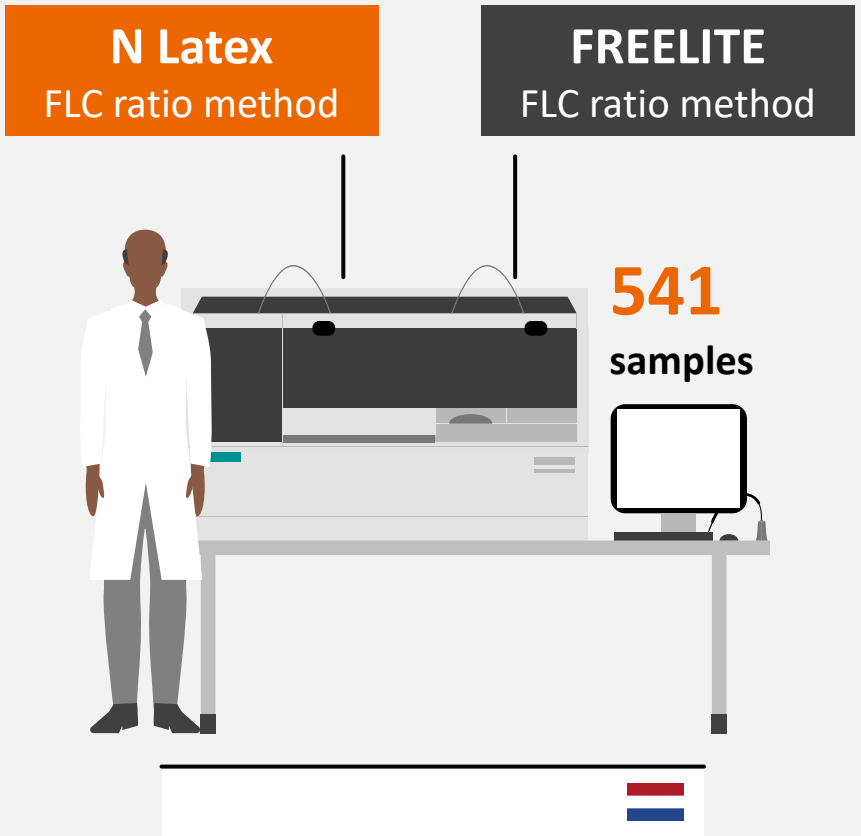


	Diagnosis	Abbreviation	Number of Samples	Number of Patients
Specificity panel	Nothing special	NS	170	165
	Renal disease	RD	154	145
	Polyclonal Ig stimulation	Poly	56	56
	Polyneuropathy	PN	1	1
Sensitivity panel	Monoclonal gammopathy of undetermined significance	MGUS	80	71
	Monoclonal gammopathy	MG	2	1
	Multiple myeloma	MM	116	63
	Kappa light chain MM	KLMM	5	3
	Lambda light chain MM	LLMM	12	6
	Kappa light chain cryoglobulin	KLCR	1	1
	Waldenström's macroglobulinemia	Wal	32	18
	Amyloidosis	AM	5	2
	Acute lymph. leukemia	ALL	1	1
	Non-Hodgkin lymphoma	NH	10	6
	Suspected of NH lymphoma	VNH	1	1
	Myelodysplastic syndrome	MDS	1	1
Total			**647**	**541**

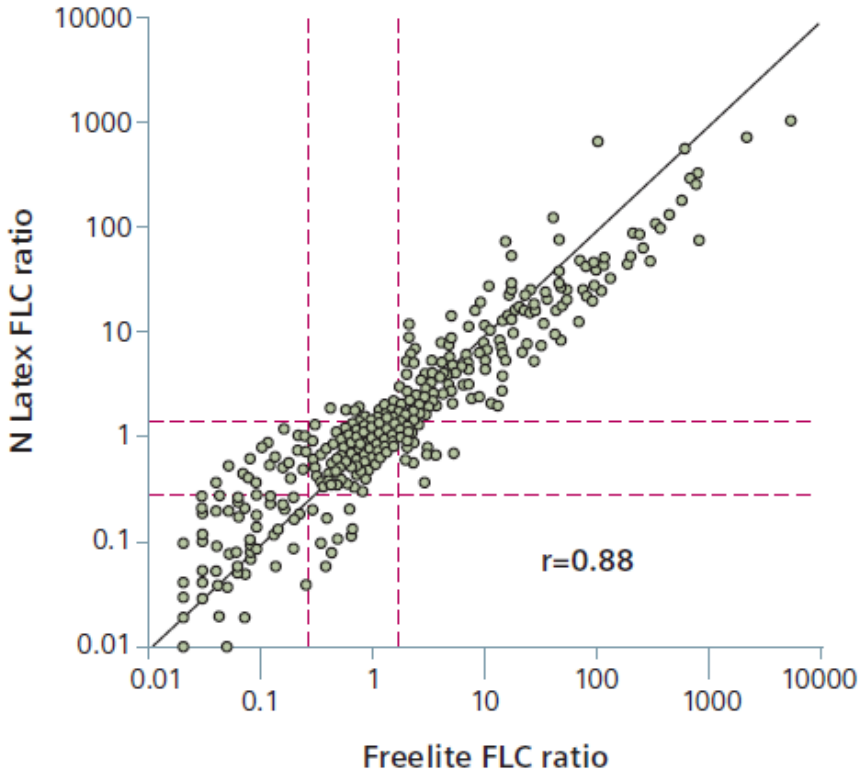
Clinical validation

Jeroen Bosch Hospital, Den Bosch, Netherlands

FLC ratio method comparison



Relatively good correlation

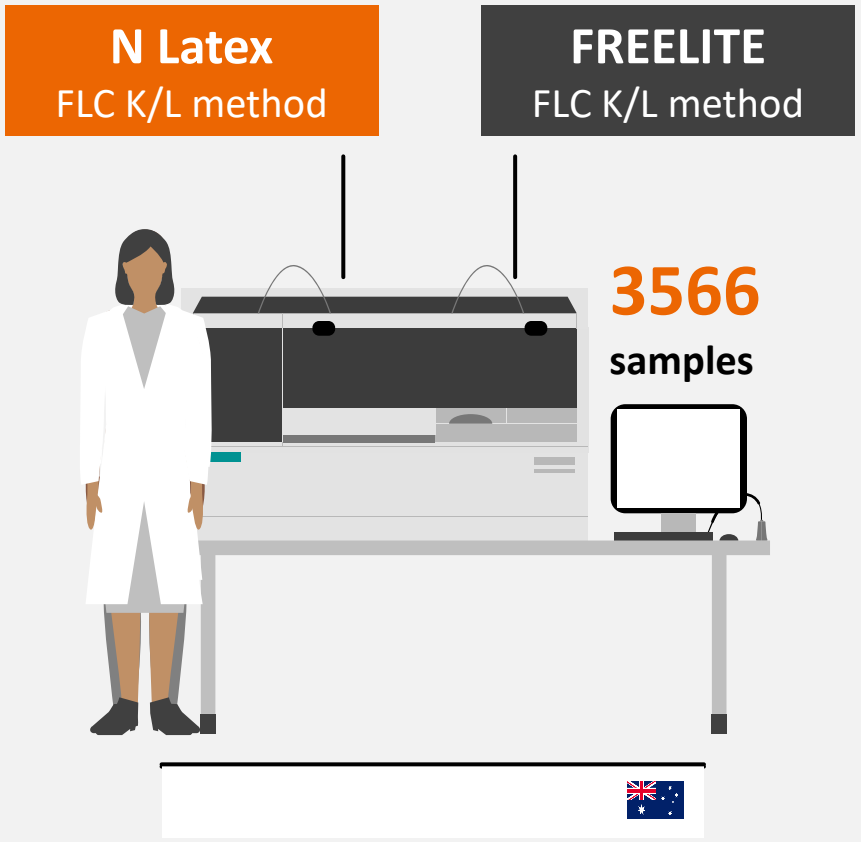


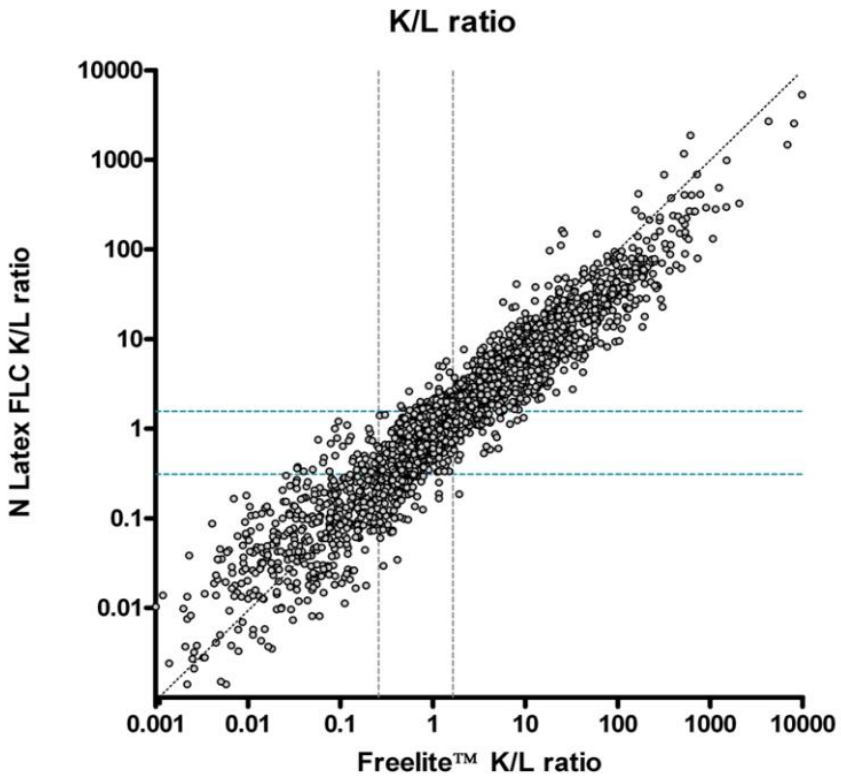
Data obtained during validation at Jeroen Bosch Hospital, Den Bosch, NL, 2010.

Clinical validation

Sullivan Nicolaides Pathology, Australia

FLC kappa/lambda method comparison



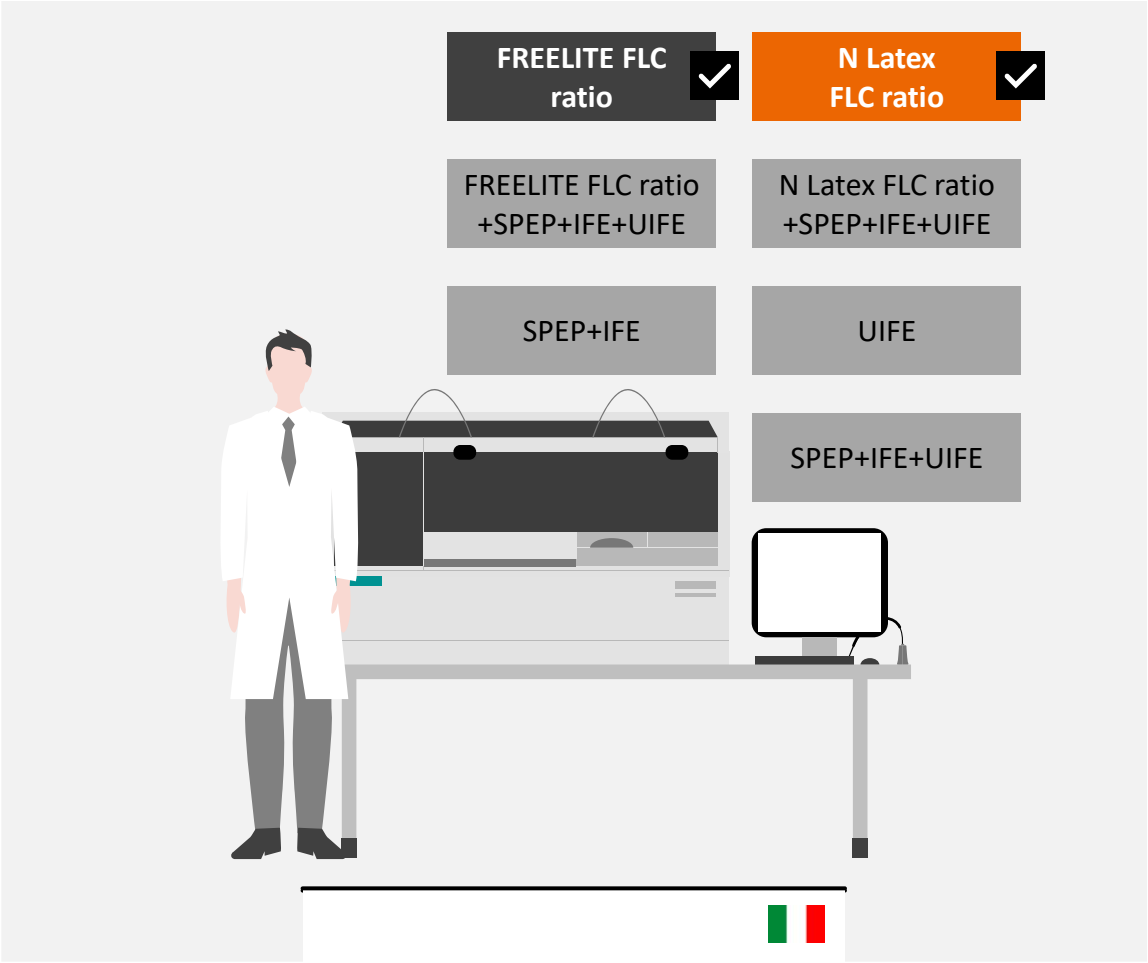


FLC comparison study at SNP, 2012.

AL amyloidosis

Ospitale di San Matteo, Pavia, Italy

Sensitivities of investigations for AL amyloidosis



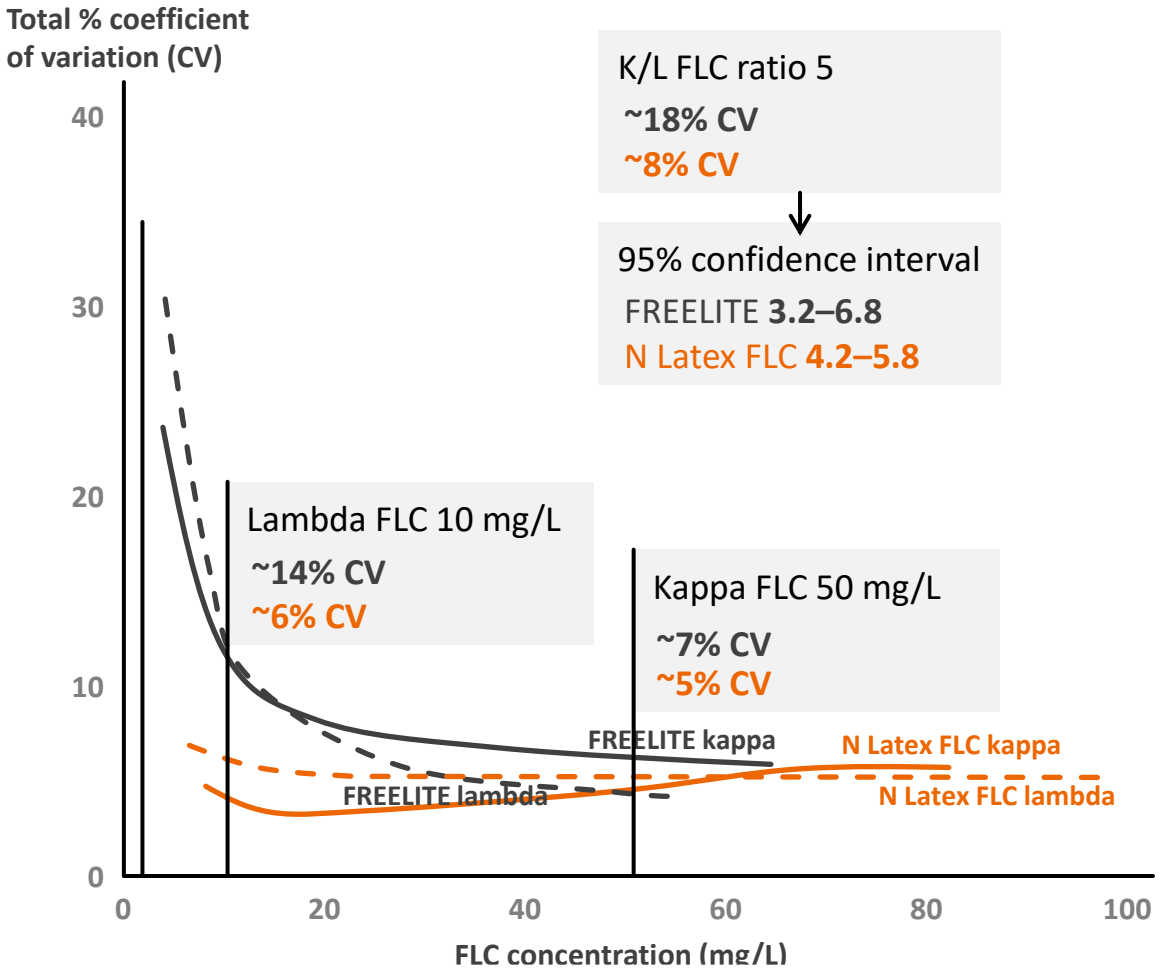
Identical results

	Pavia [13]		
	κ	λ	All
n	67	271	338
	%	%	%
SPEP+IFE	82	96	93
UIFE	81	88	87
SPEP+IFE+UIFE	84	97	94
Freelite FLC ratio	97	80	82
Freelite FLC ratio+SPEP+IFE+UIFE	100	97	98
N Latex FLC ratio	89	83	84
N Latex FLC ratio+SPEP+IFE+UIFE	95	99	98

SPEP, Serum protein electrophoresis; IFE, immunofixation electrophoresis; UEP, urine protein electrophoresis; FLC, free light chain

Mollee P, Merlini G. Free light chain testing for the diagnosis, monitoring and prognostication of AL amyloidosis. Clin Chem Lab Med. 2016 Jun 1;54(6):921-7.

RBWH: reproducibility

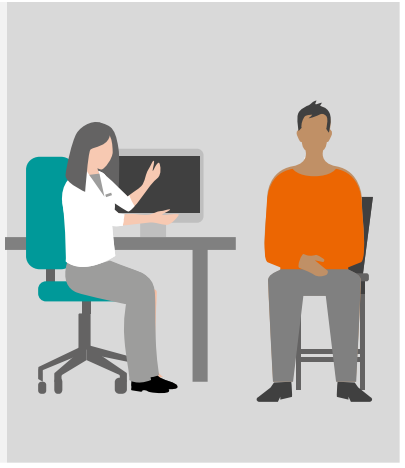


FREELITE assay's accuracy (%CV) is **variable at lower concentrations.**

Potentially inaccurate result → **Risk of Inappropriate treatment**

N Latex FLC assay's accuracy (%CV) is **steady across concentrations.**

Accurate result → **Appropriate treatment**



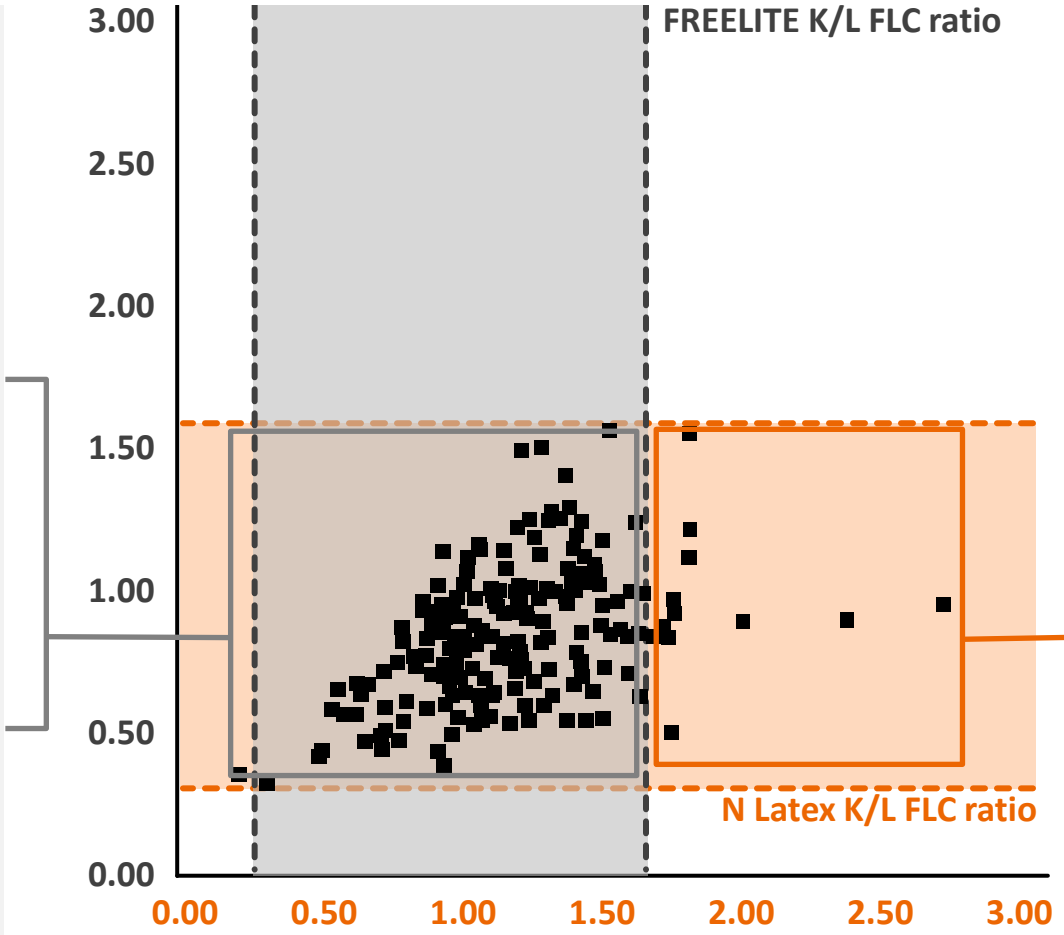
Pretorius CJ, et al. Evaluation of the N Latex FLC free light chain assay on the Siemens BN analyzer: precision, agreement, linearity and variation between lots. Ann Clin Biochem. 2012:1-6. DOI:10.1258/acb.2012.011264

RUMC, Nijmegen: FLC ratio in chronic kidney disease[1]

Certain patients' K/L FLC ratio fell **within range** when tested using N Latex and FREELITE assays.

N Latex assays
True negatives

FREELITE assays
True negatives



Other patients' K/L FLC ratio fell **within range** when testing using N Latex assays and **out of range** when tested using FREELITE assays.

N Latex assays
True negatives

Patients found to have kidney disease, **not** monoclonal gammopathy

FREELITE assays
False positives*

Some patients undergoing bone marrow biopsies to verify false positives

1. DOI 10.1515/cclm-2013-0864
*When using regular instead of specific reference range.

Overview

SIEMENS Healthineers

Multiple Myeloma: disease progression

SIEMENS Healthineers

Monoclonal Gammopathy of Undetermined Significance (MGUS)	Smoldering Myeloma (SMM)	Multiple Myeloma (MM)
No end-organ failure	No end-organ failure	Presence of end-organ failure (2 of 3 required)
Non-symptomatic	Non-symptomatic	Symptomatic
1% of patients progress per year to MM	10% of patients progress within 5 years to MM	Mean survival of 5 years[1]

First FDA-cleared free light chain assays for MGUS evaluation

Monitoring with FLC measurement

1. Goldschmidt, Deutsches Ärzteblatt No. 11/2021

IMWG and ESMO Guidelines

IMWG Guidelines published[1]

"The serum FLC assay (**FREELITE**) is based on a commercial reagent set of polyclonal antibodies and is performed by **immunonephelometry** and it can be performed on a number of automated laboratory instruments."[1]

IMWG Guidelines updated[2]

"The free light chain (FLC) assay is an automated **nephelometric** assay that identifies and measures κ and λ light immunoglobulin chains that circulate unbound to heavy chains in the serum."[2]

European Society for Medical Oncology **(ESMO) Guidelines** published[3]

"Diagnosis of MM should be based on the following tests: Detection and evaluation of the monoclonal (M) component by serum and/or urine protein electrophoresis (concentrate of 24h urine collection); **nephelometric quantification of IgG, IgA and IgM immunoglobulins**; characterisation of the heavy and light chains by immunofixation; and **serum-free light-chain (FLC) measurement**."[3]

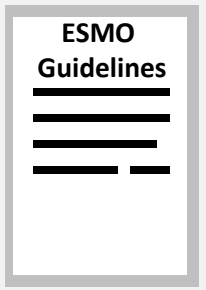
FREELITE mentioned specifically

No specific brand mentioned

No specific brand mentioned

2009 **2010** **2014** **2017**

Siemens Healthineers assays launched



1. Dispenzieri, et al. IMWG guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia. 2009;23:215-24.
2. Rajkumar, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538-48.
3. Moreau, et al. Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2017;28(Suppl 4):iv52–iv61.

Multiple myeloma: predicting the likelihood of progression

Study carried out using N Latex FLC assays

Monoclonal gammopathy of undetermined significance (MGUS)

1% of patients with MGUS progress per year to MM

Patients with **no risk factors** have a

2% 5-year cumulative probability of progression

Patients with **2 or 3 risk factors** have a

31% 5-year cumulative probability of progression

Risk factors determining likelihood of progression[1]

01 | **M-spike**
>1.5 g/dL

02 | **Age**
>65 years

03 | **iFLC**
>100 mg/L

Multiple myeloma

1. Gran, at al. Comparative evaluation of involved free light chain and monoclonal spike as markers for progression from monoclonal gammopathy of undetermined significance to multiple myeloma. Am J Hematol. 2021;96:23-30.

Multiple myeloma: predicting the likelihood of progression

Revised list of diagnostic criteria for SMM and MM: Rule 100[1]

Both **N Latex FLC** and **FREELITE** assays accurately identified **high-risk** and **ultra-high-risk** SMM patients.

Smoldering myeloma

10% of patients with SMM progress per year to MM

Rule 100 criteria

Clonal bone marrow plasma cells ≥10%

+

01 | Clonal bone marrow plasma cells ≥60%

02 | **Involved: uninvolved sFLC ratios ≥100**

03 | >1 focal lesions on MRI studies

Multiple myeloma

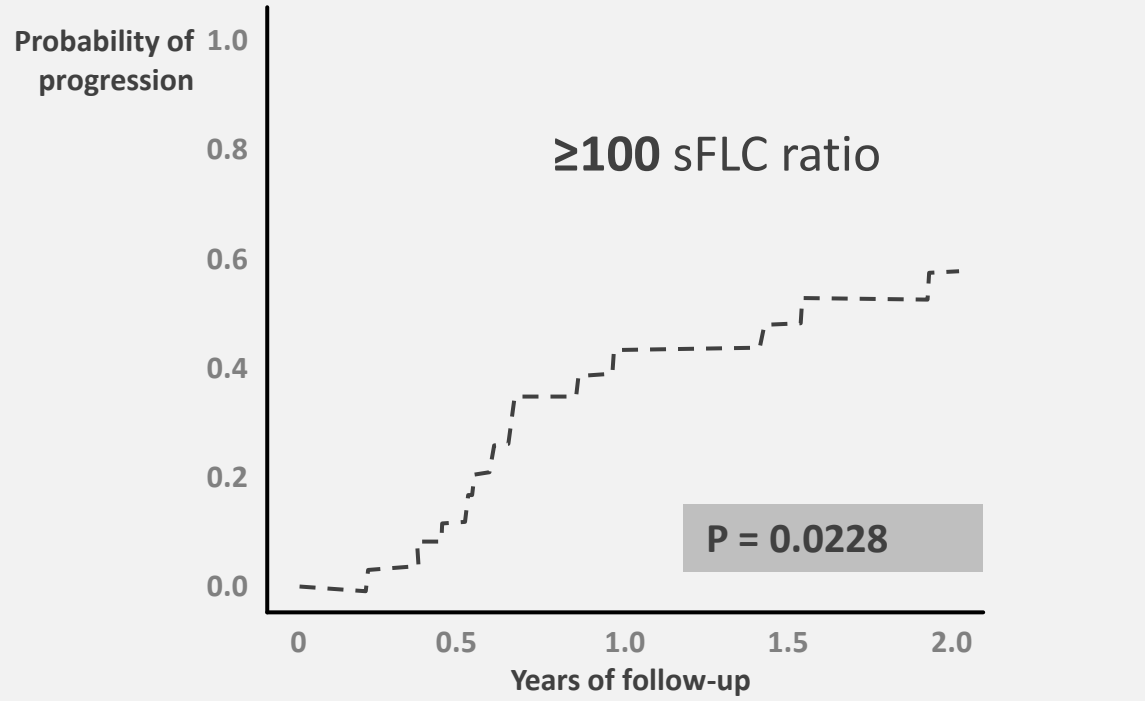
1. Rajkumar, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538-48.

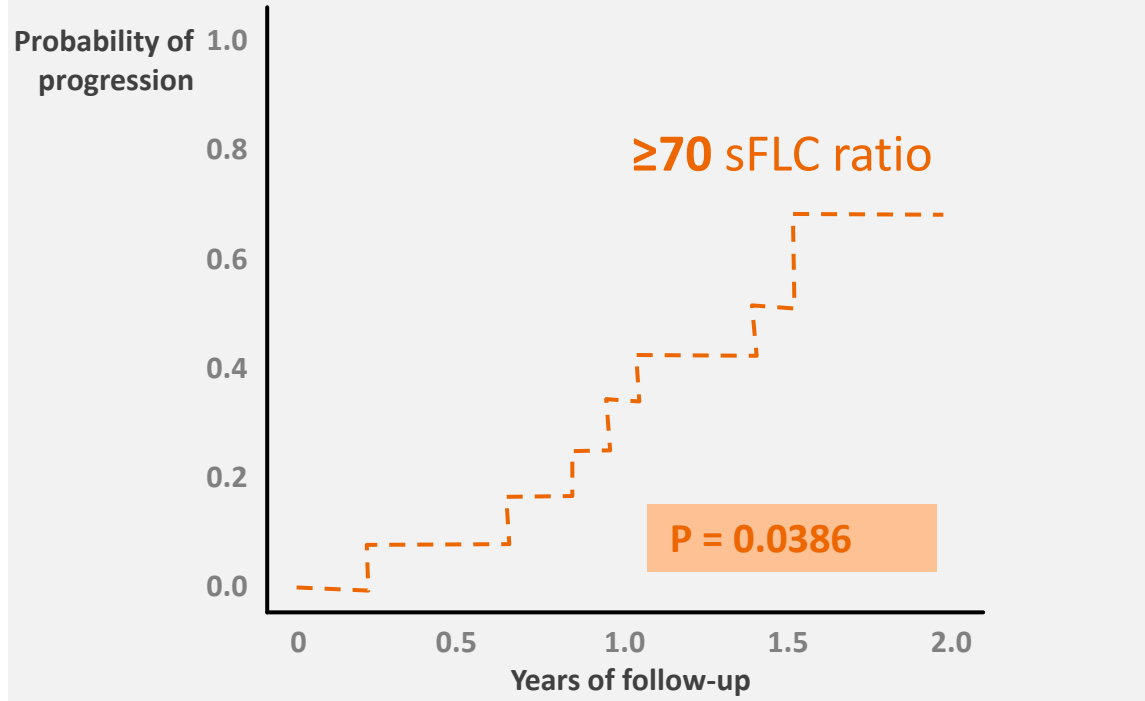
Rule 100: further discussion points

N Latex assays can be used for SMM monitoring, like FREELITE assays

FREELITE Assays	N Latex FLC Assays

N Latex assay ≥70 sFLC ratio provides **similar performance** to a FREELITE assay sFLC ratio ≥100, with **a slightly better** positive predictive value.[1]





1. Henriot, et al. Prognostic value of involved/uninvolved free light chain ratio determined by Freelite and N Latex FLC assays for identification of high-risk smoldering myeloma patients. Clin Chem Lab Med. 2019. https://doi.org/10.1515/cclm-2018-1369

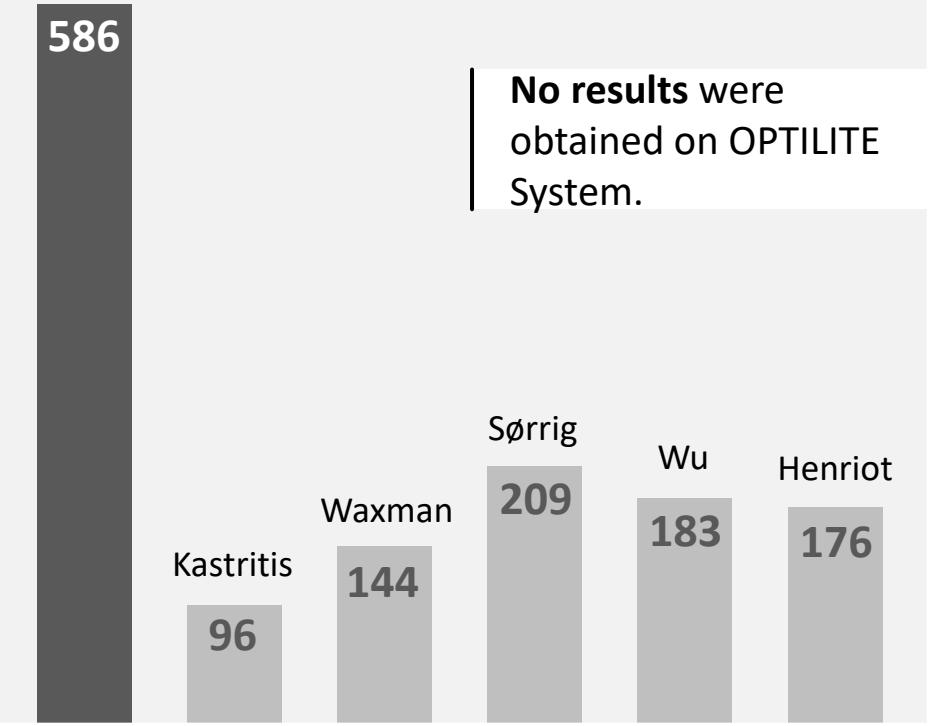
Rule 100: clinical evidence from published data

SIEMENS Healthineers

It has not always been possible to repeat Larson's initial findings.

	PPV	Specificity
2013 **Larson**	73%	97%
2013 **Kastritis**	43%	98%
2013 **Waxman***	64%	NA
2016 **Sørrig**	30%	NA
2018 **Wu**	44%	90%
2019 **Henriot** (FREELITE assay)	57%	91%
2019 **Henriot** (N Latex FLC assay)	67%	97%

Larson tested **many more samples** than were tested in any other study.



No results were obtained on OPTILITE System.

*18-month outcome. All other studies were 24 months.

2/20/20+ risk stratification model

International Myeloma Working Group (IMWG) 2020

SIEMENS Healthineers

IMWG objective:

Develop a risk stratification model to identify SMM patients at **high risk of progressing to MM**, or other plasma cell disorders.[1]

Smoldering myeloma

Four independent factors[2], predicting progression risk at 2 years since diagnosis

01	Serum M-protein **>2 g/dL** (HR: 2.1)
02	Involved: uninvolved FLC ratio **>20** (HR: 2.7)[3]
03	Marrow plasma cell infiltration **>20%** (HR: 2.4)
04	Cytogenetic abnormalities

Multiple myeloma

✓ Helps to identify **high-risk SMM patients**

✓ **Widely applicable**

1. 50% progression risk within first two years from diagnosis. Based on the 2014 IMWG criteria for definition of both SMM and MM.
2. Mateos, et al. International Myeloma Working Group risk stratification model for smoldering multiple myeloma (SMM). Blood Cancer Journal. 2020;10:102.
3. Neither FREELITE or any other brand name is mentioned regarding FLC testing.

Risk factors and progression risk from SMM to MM

Within first 2 years from diagnosis

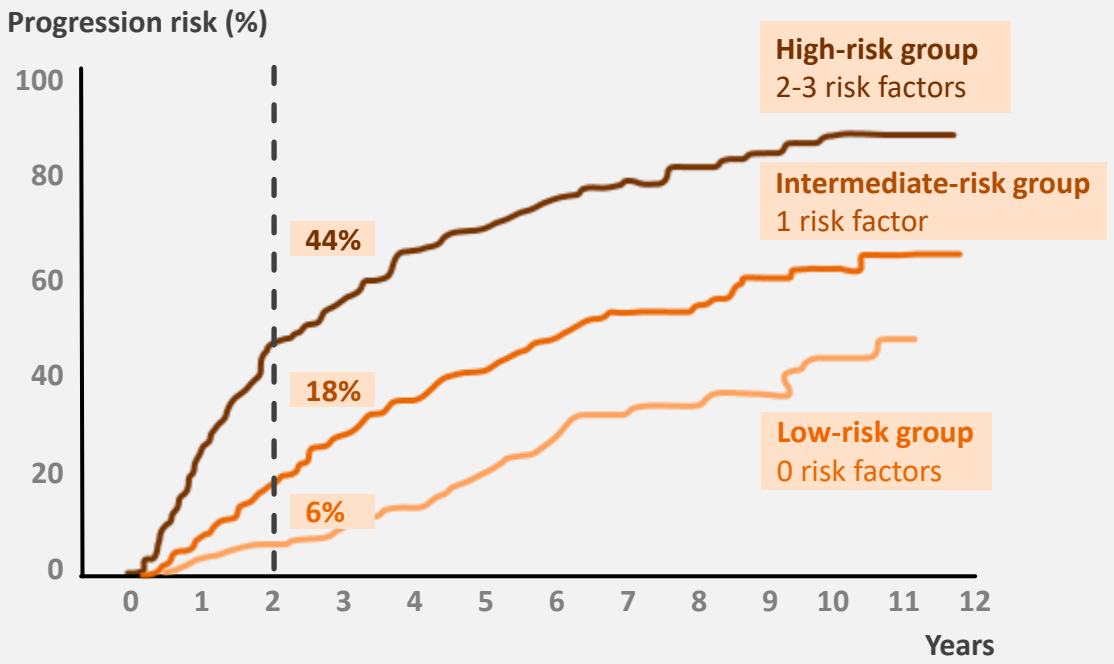
01 Serum M-protein **>2 g/dL** (HR: 2.1)

02 Involved: uninvolved FLC ratio **>20** (HR: 2.7)

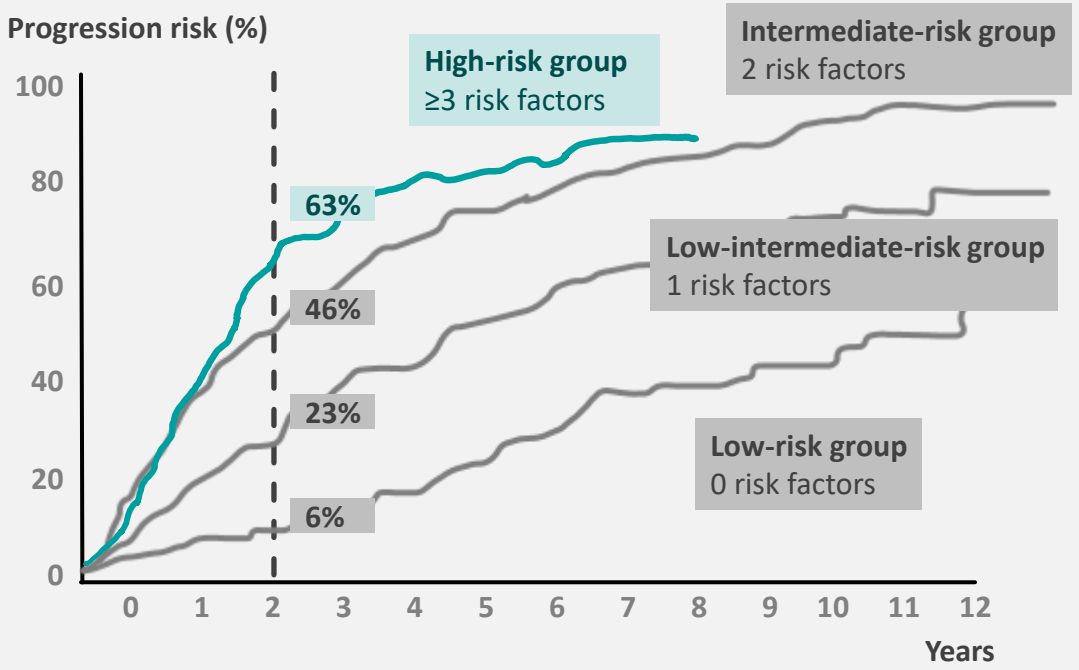
03 Marrow plasma cell infiltration **>20%** (HR: 2.4)

04 Cytogenetic abnormalities

2/20/20 Model: **three risk factors**



2/20/20+ Model: **four risk factors** (also accounting for cytogenetic abnormalities*)



Higher-risk patients identified earlier when cytogenetic abnormalities included in risk criteria.

*Cytogenetic abnormalities present (e.g., t(4;14), t(14;16), +1q, del13q/monosomy 13 by (FISH)).

Overview

SIEMENS Healthineers

01 Basics

02 Difference from competition

03 Performance comparison

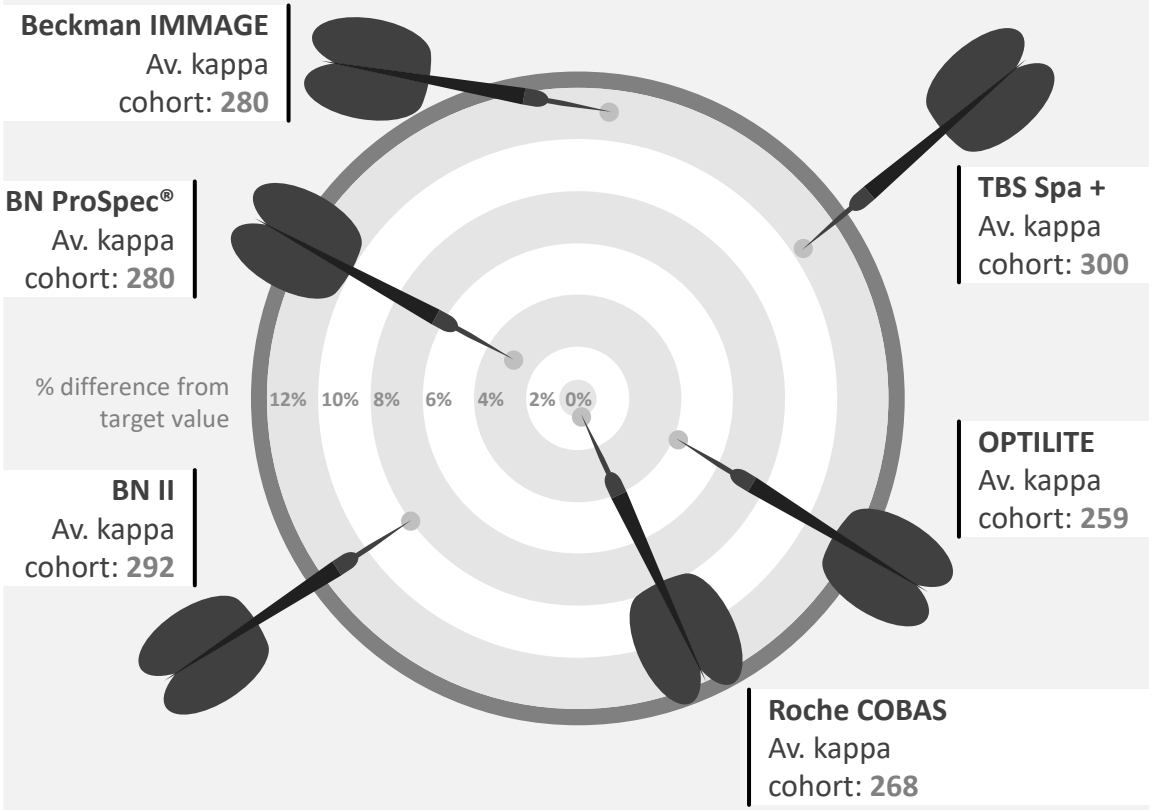
04 Guidelines and risk stratification

05 Implementation

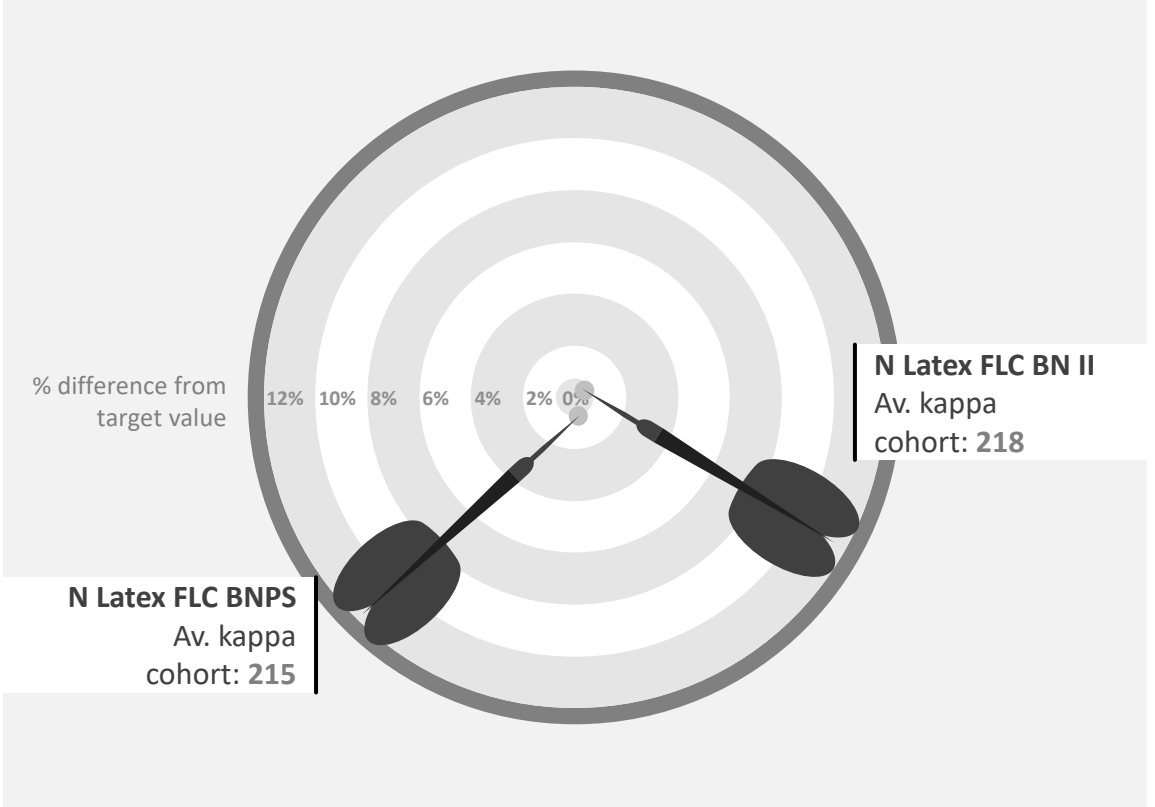
Switching to a new assay

Comparison of serum free light chain assays on different systems by UK NEQAS

FREELITE assay – kappa target value: 271



N Latex FLC assay – kappa target value: 218

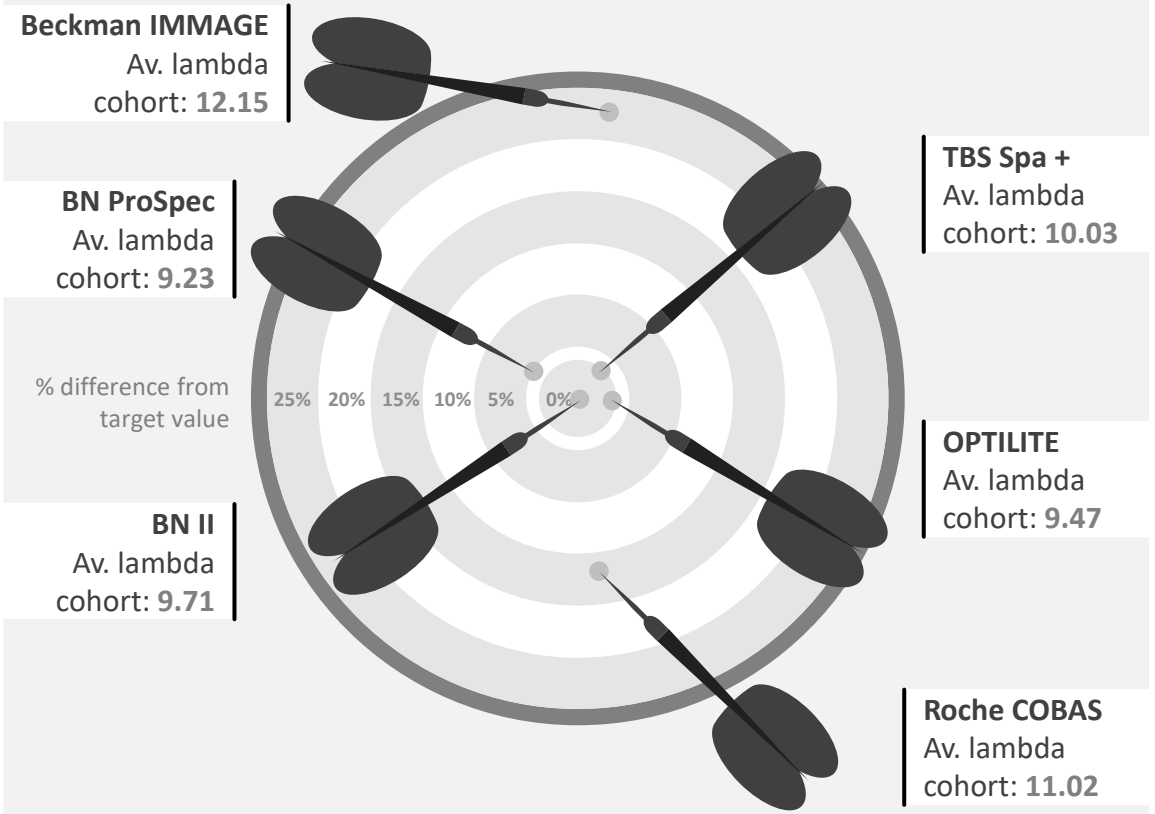


UK NEQAS Report for Monoclonal Proteins 201, 25-Feb-2020.

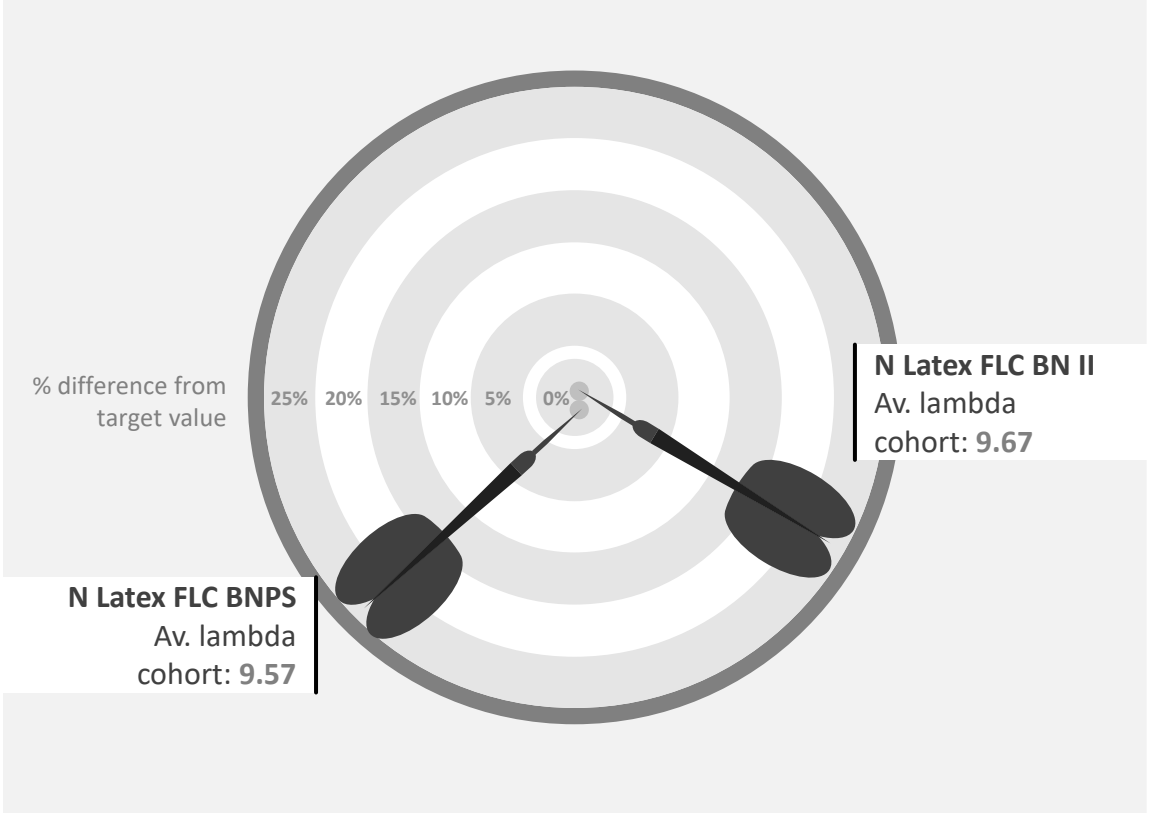
Switching to a new assay

Comparison of serum free light chain assays on different systems by UK NEQAS

FREELITE assay – lambda target value: 9.75



N Latex FLC assay – lambda target value: 9.63



UK NEQAS Report for Monoclonal Proteins 202, 05-May-2020.

Switching to a new assay

FDA also concurs that...

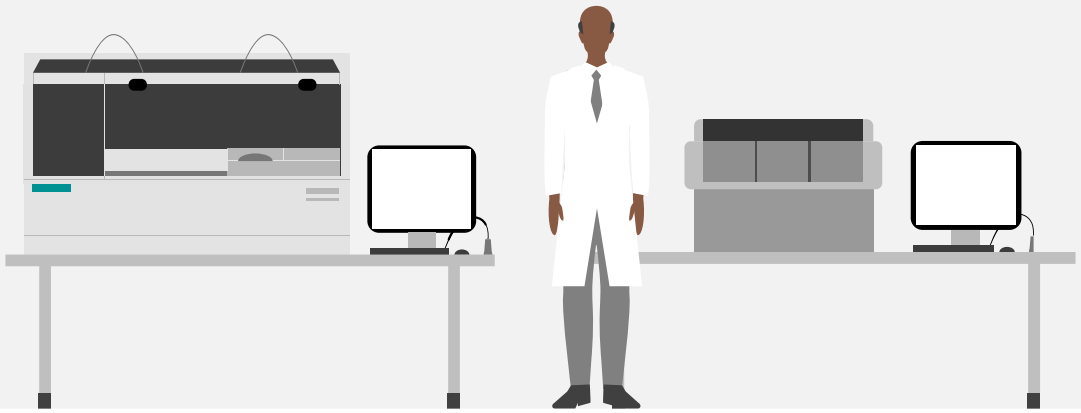
*"Prior to changing assay or system, the laboratory **MUST** confirm baseline values for patients being serially monitored."*[1]

Switching to a new assay

FREELITE assay → Baselining required → N Latex FLC assay

Switching to a new instrument

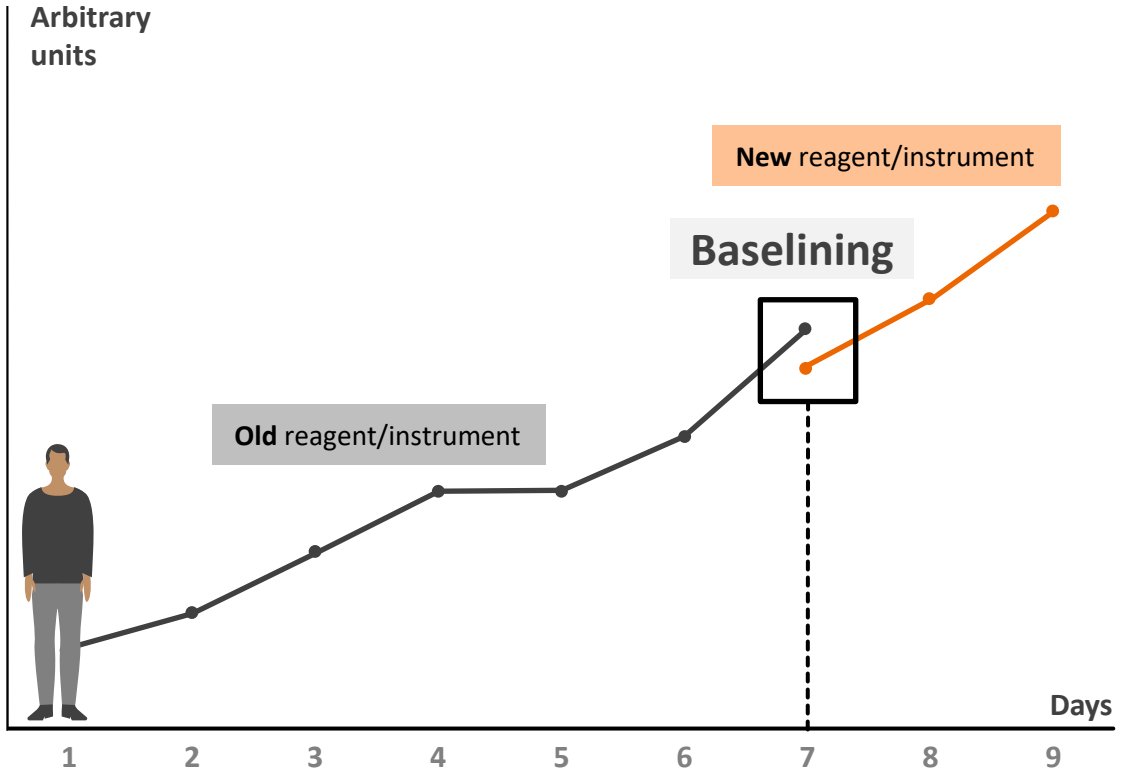
BN II → Baselining required → OPTILITE



1. Substantial Equivalence Determination Decision Memorandum, 510(k) Number K150658 for The Binding Site Group, Ltd., 08/26/2015.

Baselining: comparable results

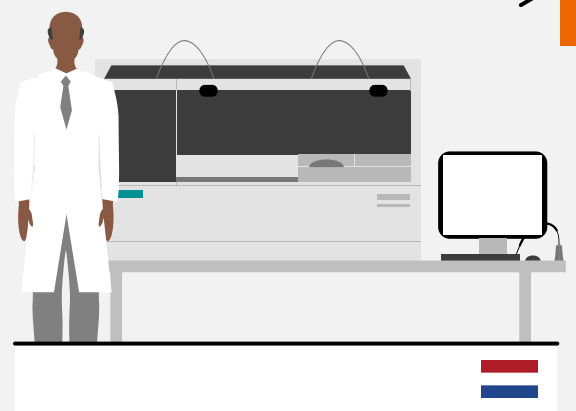
Jeroen Bosch Hospital, Den Bosch, Netherlands



Patient's sample measured for last time using old system and measured **in parallel** using new system.

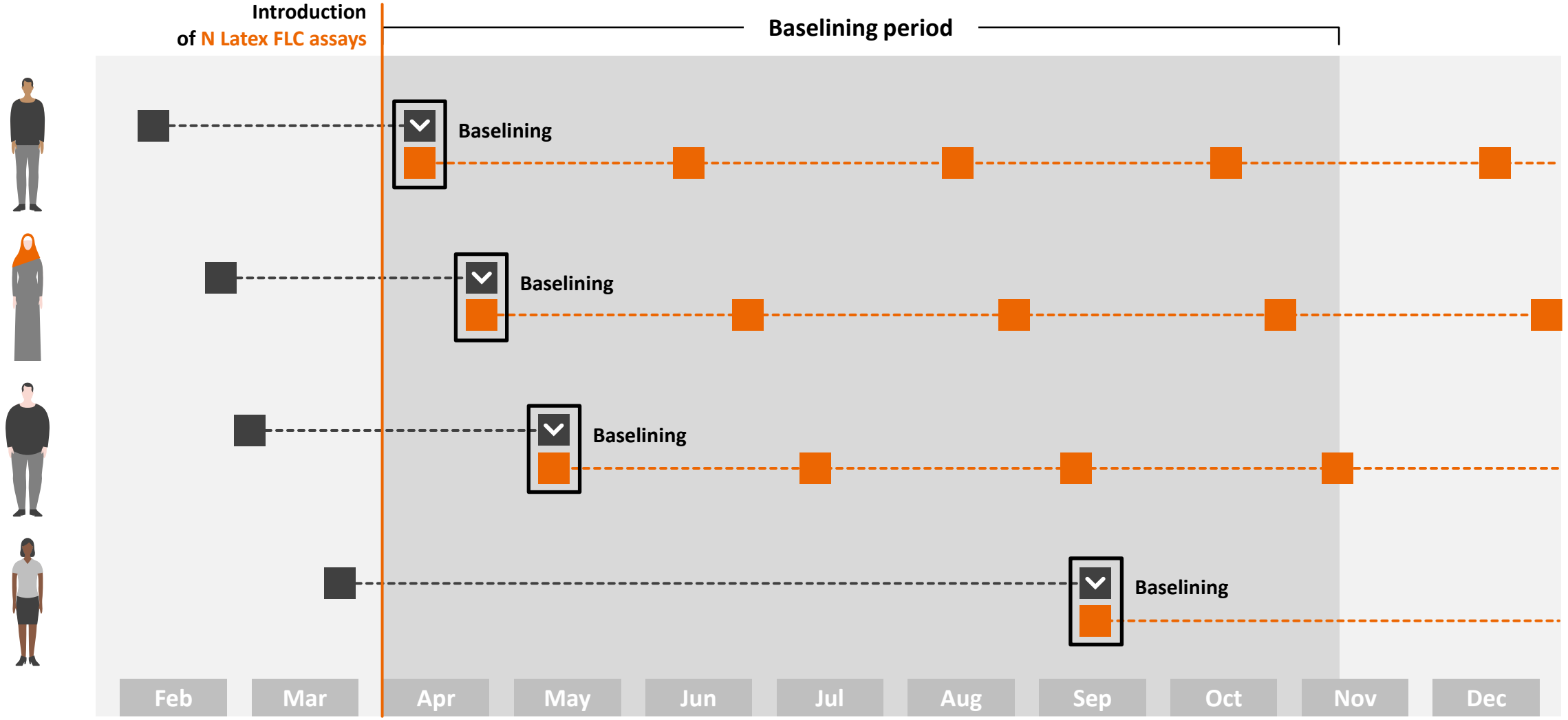
FREELITE Kappa	5.4	7.8	9.8	7.7	6.8	7.2	**7.2**		
FREELITE Lambda	180	270	410	550	550	680	**930**		
Ratio K/L	0.03	0.029	0.024	0.014	0.012	0.011	**0.0077**		
N Latex FLC Kappa							**7.9**	4.7	8.7
N Latex FLC Lambda							**940**	1100	1300
Ratio K/L							**0.0084**	0.0043	0.0067

FREELITE assay → **N Latex FLC assay**



Patient baselining in practice

Patients are monitored on different days, and so have staggered baselining periods.



Key takeaways

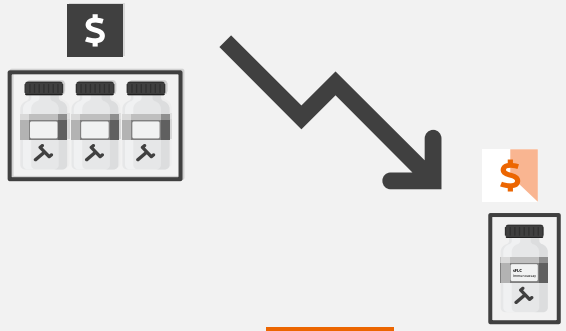
N Latex FLC kappa and lambda assays are:

01 As effective as FREELITE assays

Equivalent results

No risk of false-positive results for patients with chronic kidney disease.

02 More flexibility than FREELITE assays



Flexible monoclonal reagent kit

Order components when you need them, rather than buying an entirely new kit each time.

03 Baselining required

When switching reagents

And when switching instruments.

N Latex FLC assays

?

Any questions?