Standardization and Metrological Traceability of a Commercial anti-MAG (Myelin Associated Protein) Antibodies ELISA

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INTRODUCTION

The BÜHLMANN anti-MAG Antibodies ELISA (MAG-ELISA) is the acknowledged gold standard *in vitro* diagnostic ELISA, to reliably quantify anti-MAG IgM antibodies in demyelinating neuropathies, a rare autoimmune-disease. There are no recognized reference materials or reference measurement procedures for anti-MAG antibodies. We guarantee measurement consistency for the end-users over time, with a transparent traceability chain. This is achieved by using an internal reference material (IRM) to produce standardized calibrators (Fig. 3). The anti-MAG antibodies ELISA is therefore ready for IVDR, the new European regulatory basis for in vitro diagnostic medical devices.

METHODS

An IRM is generated from pooled individual patient sera, which are positive for anti-MAG IgM antibodies. Following the protocol by Blirup-Jensen et al., 2008 (Fig. 3), the value of the IRM is assigned to a calibrator stock, which is subsequently gravimetrically diluted into calibrators (Fig. 2). The IRM traceable MAG-ELISA was compared to the current version (Fig. 4). In addition, within-laboratory precision (total), repeatability (within run) and reproducibility (total) were assessed, measuring five samples covering the measurement range. Within-laboratory precision (total) and repeatability (within-run) were assessed with 20 days * 2 runs * 2 replicates (n_{tot} = 80), reproducibility (total) with 3 instruments/ lots/ operators con-founded * 5 days * 1 run * 5 replicates (n_{tot} = 75).
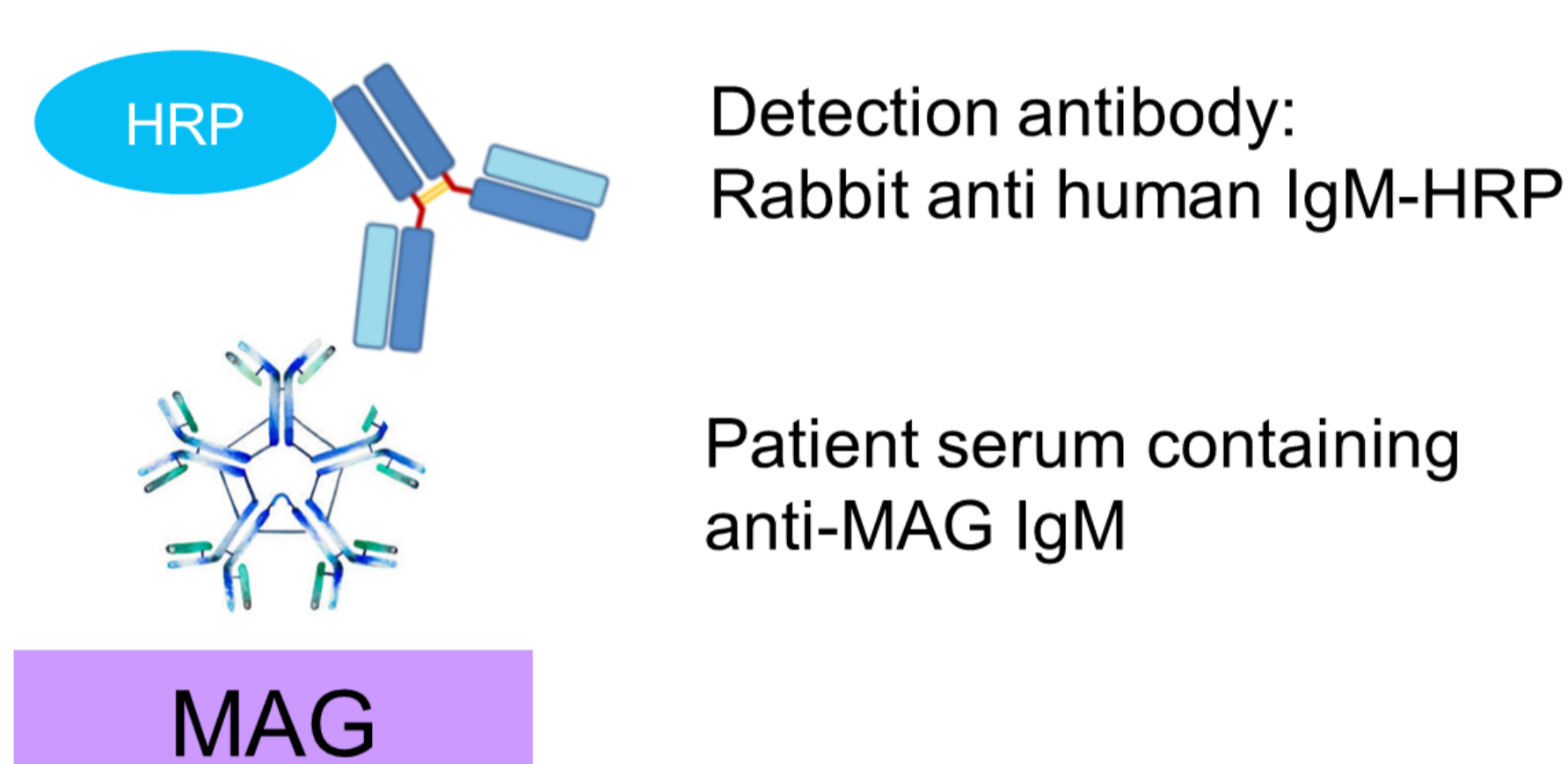
RESULTS

The combined uncertainty of the calibrator at the 95% confidence interval is 31% (Fig. 3).
The IRM traceable calibrators demonstrate acceptable trueness when compared to the current calibrator material: the slope in the Passing-Bablok was 0.97 and a mean bias of 8.1% was determined for the measuring range of the MAG-ELISA (Bland-Altman analysis) (Fig 4).
The within-laboratory precision (total) %CV was less than 20% with a range of 5.5 - 15.9%. The repeatability (within run) %CV was less than 15% with a range of 3.2 - 11.8% (Fig. 5).
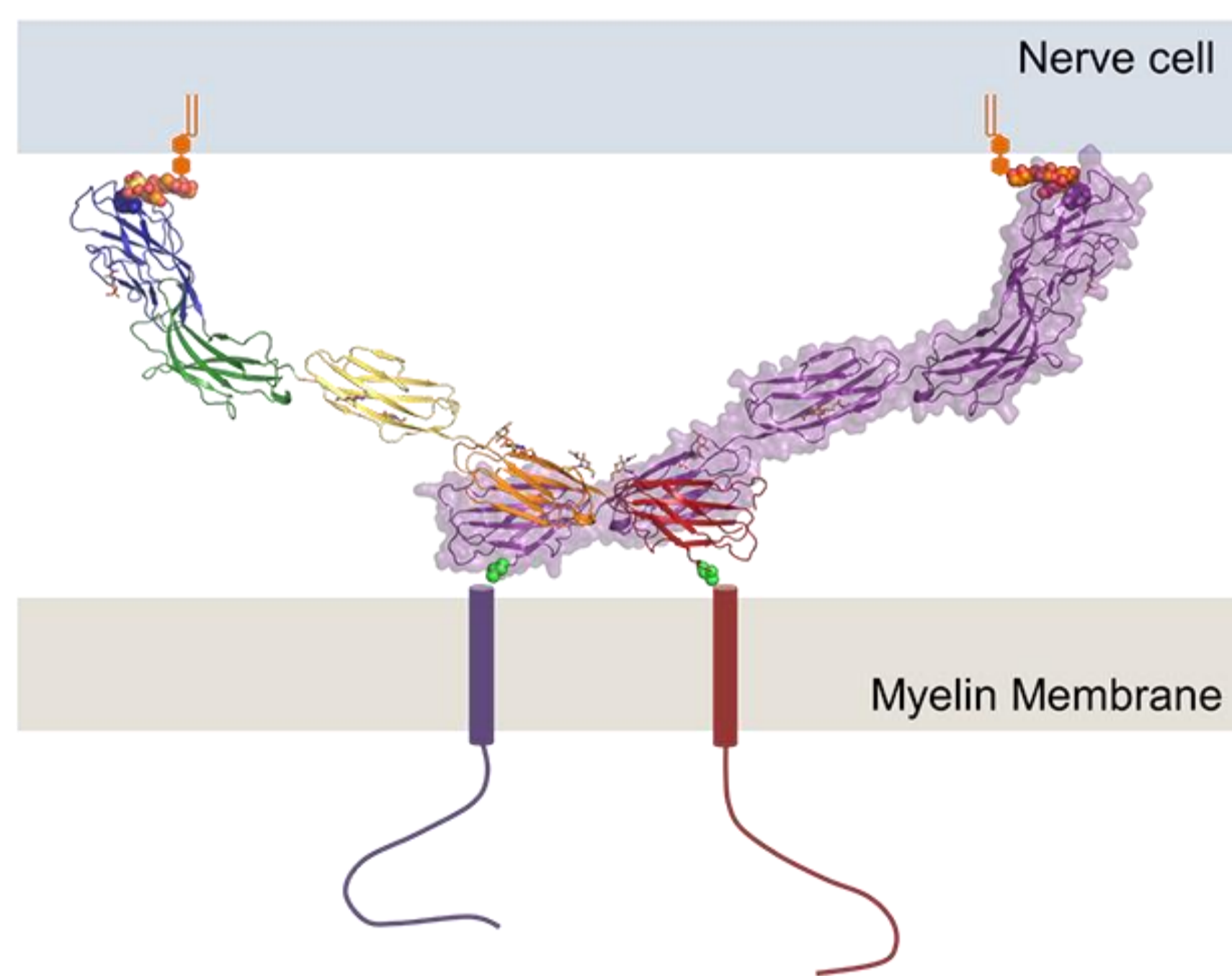The reproducibility (total) %CV was less than 25% with a range of 10.0 - 21.6% (Fig. 6).

FIGURES

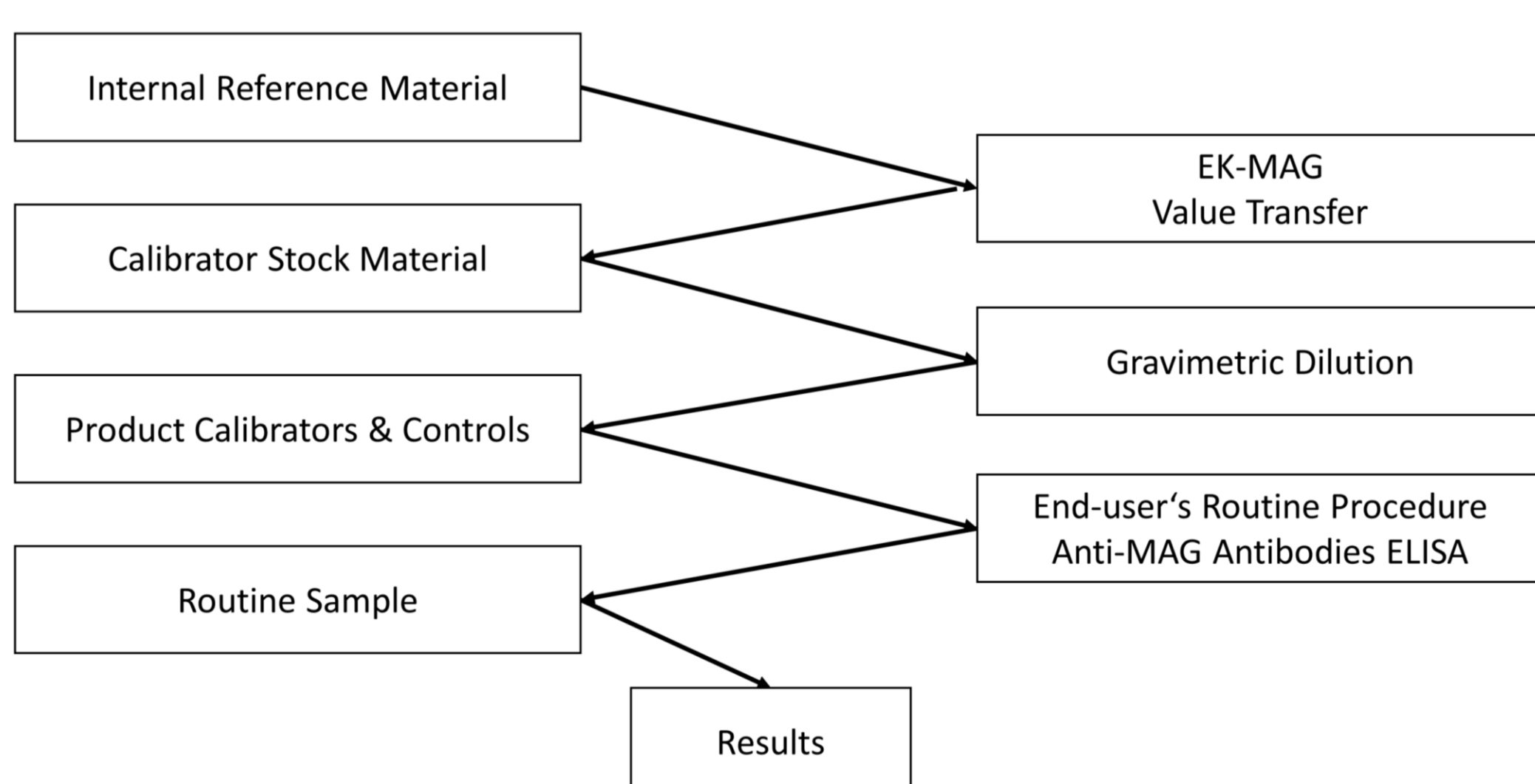### Figure 1:

a) Principle of the assay: sandwich ELISA

Detection antibody:
Rabbit anti human IgM-HRP

Patient serum containing anti-MAG IgM

MAG

b) Myelin Associated Glycoprotein

Nerve cell

Myelin Membrane

Pronker et al, 2016 Nature Communications Doi: 10.1038/NCOMMS13584

Figure 2: Metrological Traceability & Standardization

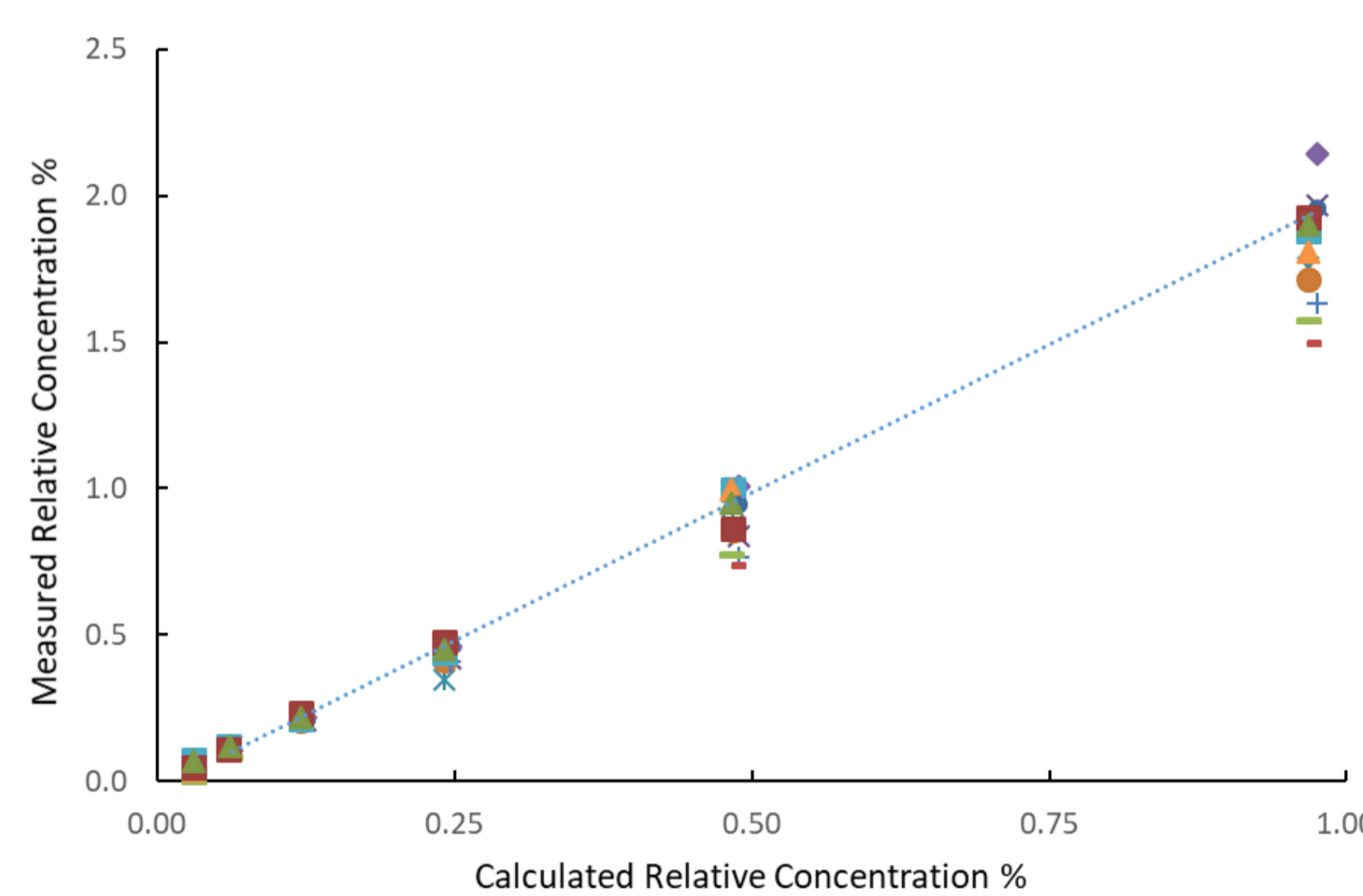CLSI Guideline EP32-R Metrological Traceability and Its Implementation; A Report
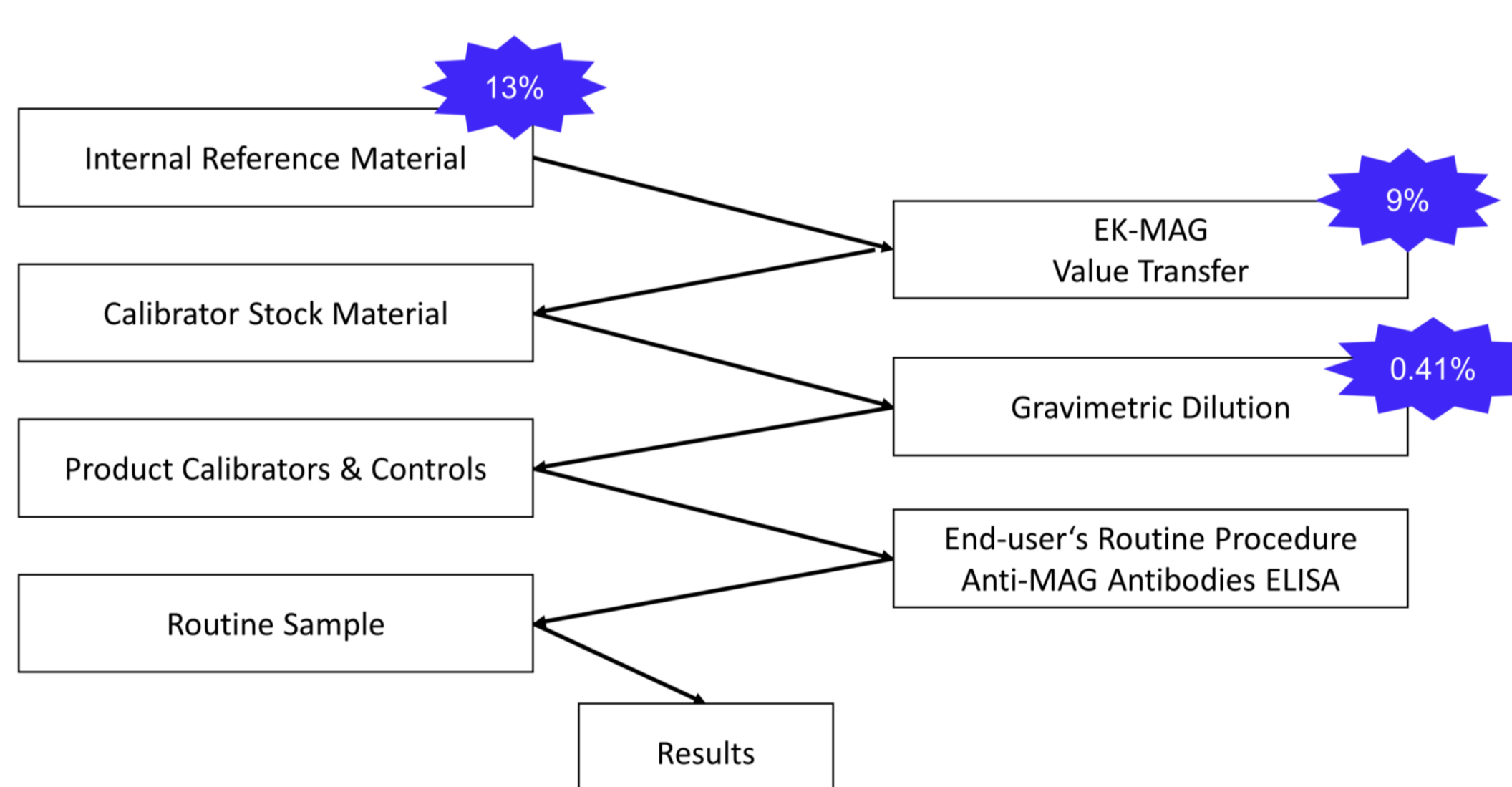
Figure 3:

a) Value Transfer Protocol

	# dilutions	# runs	# replicates	# days
IRM	6	3	2	4
Calibrator Material	5 - 6	3	2	4
IRM Mastercontrol	1	3	2	4

IRM = Internal Reference Material

b) Value Assignment Calibrator Stock

c) Uncertainty Calculation

$$U_{comb} \text{ of Calibrator at 95\% CI} = 2x\sqrt{u_{IRM}^2 + u_{Value\ Transfer}^2 + u_{CASET\ Dilution}^2} = 2x\sqrt{13^2 + 9^2 + 0.41^2} = 31\%$$

The combined uncertainty of the calibrator at the 95% CI is 31%.

Blirup-Jensen et al., 2008, DOI 10.1515/CCLM.2008.289

Figure 4: Current assay vs. IVDR assay

The IRM traceable MAG-ELISA was compared to the current version. The slope in the Passing-Bablok was 0.97 and the mean bias in the Bland-Altman was 8.1%.
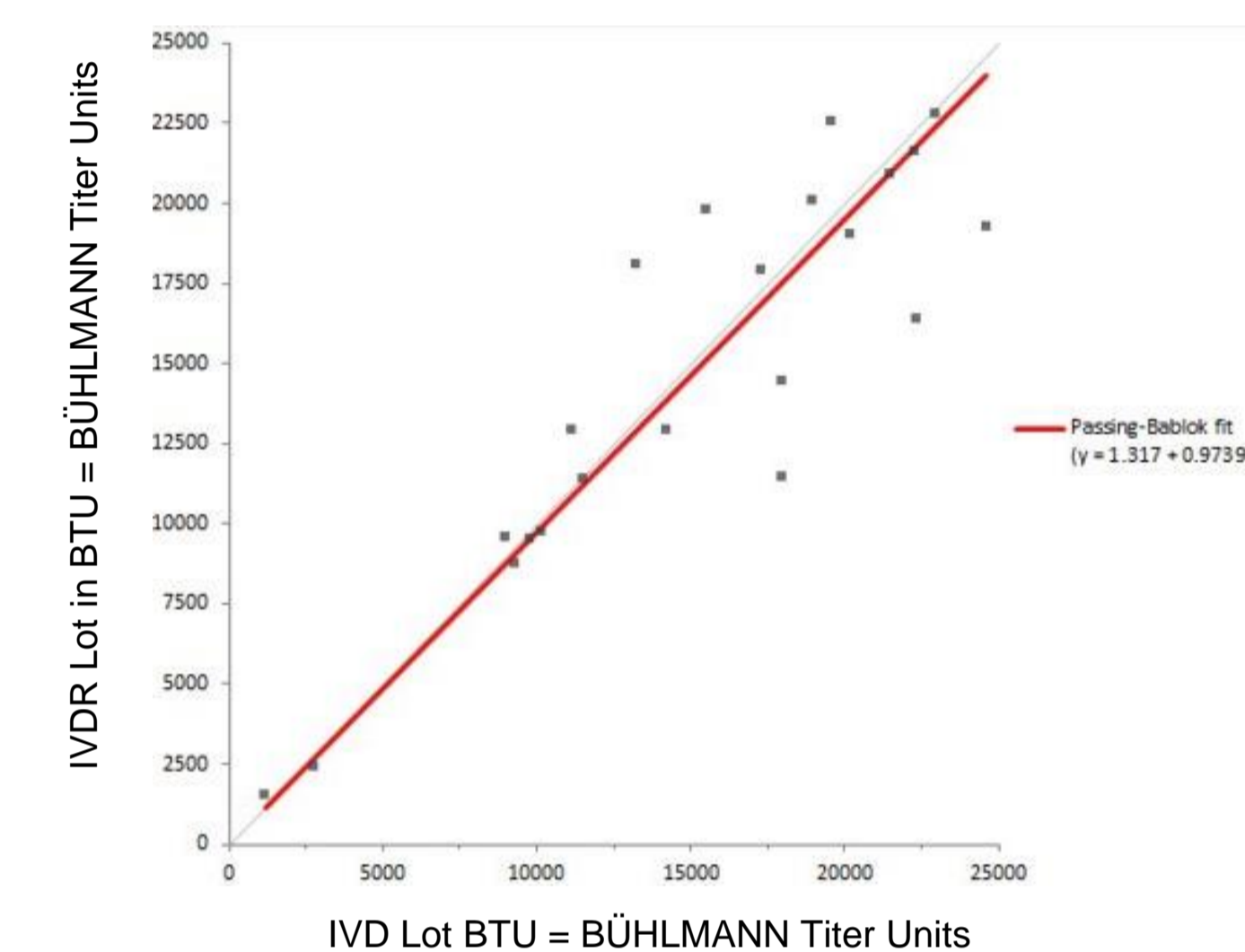

Figure 5: Within-laboratory precision (total) and repeatability (within run) :
20 days * 2 runs * 2 replicates (n total = 80)

Sample	Mean, BTU	N	Within-Run		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
2	2250.7	80	266.6	11.8	198.5	8.8	130.3	5.8	357.0	15.9
3	8849.3	80	348.7	3.9	314.3	3.6	121.9	1.4	485.0	5.5
4	19683.3	80	622.1	3.2	1492.5	7.6	908.4	4.6	1854.6	9.4
5	37185.0	80	1683.7	4.5	3082.5	8.3	1465.5	3.9	3805.9	10.2

The within-laboratory precision (total) %CV was less than 20% with a range of 5.5 - 15.9%.
The repeatability (within run) %CV was less than 15% with a range of 3.2 - 11.8%

Figure 6: Reproducibility
3 instruments/ lots/ operators * 5 days * 1 run * 5 replicates (n total = 75).

Sample	Mean, BTU	N	Within-Run		Between-Day		Between-Lot-Inst.-Operator		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
2	2802.0	75	180.8	6.5	516.6	18.4	260.8	9.3	606.3	21.6
3	9051.8	75	258.2	2.9	820.7	9.1	278.7	3.1	904.4	10.0
4	18240.7	75	530.9	2.9	1146.3	6.3	1474.6	8.1	1941.8	10.6
5	34713.4	75	893.5	2.6	2740.0	7.9	2023.4	5.8	3521.4	10.1

The reproducibility (total) %CV was less than 25% with a range of 10.0 - 21.6%.

CONCLUSIONS

The transparent traceability chain of the anti-MAG Antibody ELISA not only truly displays the assay's uncertainty and leads to stable results, but also **confirms its gold standard status**.

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All authors are employees of BÜHLMANN Laboratories AG.