How do I

quantify anti-HPA-1a antibodies?

Prepared by:

Gerald Bertrand

Blood Center of Brittany, Platelet Immunology Laboratory
Rennes, France

Maria Therese Ahlen

Norwegian National Unit for Platelet Immunology,
University Hospital of North Norway, Tromsø, Norway

Lucie Richard

Héma-Québec, Stem Cells and Reference Laboratory,
Saint-Laurent, Quebec, Canada

Detection and quantitation of anti-HPA-1a

How do I

Anti-HPA-1a is the most common alloantibody relevant for FNAIT, and several studies have documented the correlation of anti-HPA-1a antibody levels and its effect on clinical outcome. (Kjaer et al. *Vox Sang* 2018)

MAIPA is the most commonly used method, but detailed MAIPA protocols vary among laboratories (ISBT platelet Workshop report 2018).

There is yet no international consensus on how to measure and to use the anti-HPA-1a antibody levels during pregnancy for risk and treatment management.
Therefore, the offer of quantitative MAIPA as a clinical laboratory test varies, depending on the national recommendations for risk management and/or experience with the potential benefit of the test.

Anti-HPA-1a standards

An official anti-HPA-1a standard (100 IU) is available from NIBSC code 03/152 (UK).
The standard is of limited source and intended for calibration for clinical laboratories.
In-house standards (serum or mAbs) calibrated against the NIBSC are also used.

How do I… or How do we…

Since protocols and clinical use of quantitative MAIPA for anti-HPA-1a varies among laboratories, we here present protocols from two different laboratories as examples in a comparative manner.

How do I

	BloodCenter of Brittany, France	Norwegian National Unit for Platelet Immunology
Mouse monoclonal Ab against αIIb/β3	Clone P2 (anti-CD41)	Clone Y2/51 (anti-CD61)
Standard	NIBSC 03/152	NIBSC 03/152 mAb 26.4 and in-house plasma
Dilutions of standard	1:64 – 1:512	1:8 - 1:256
Anti-HPA-1a antibody levels reported	IU/mL	IU/mL
Sample material	Serum	EDTA plasma
Interpretation of results	Excel file originally designed by the NIBSC	In house

How do I… or How do we…

ISBT How do I

	BloodCenter of Brittany, France	Norwegian National Unit for Platelet Immunology
Correction factor for haemodilution during pregnancy	Yes, according to the term of gestation (Bertrand et al. *JTH* 2006)	No correction factor used
Interference seen with anti-HLA class I antibodies?	Yes	Yes
How is this managed?	Chloroquine-treated platelets if very strong (for samples and reference)	Most often resolved by sample dilution
Do you follow the antibody concentration during pregnancy?	Yes: During pregnancy and At delivery (Bertrand et al. *Blood* 2014)	Yes: During pregnancy and At delivery (Tiller et al. *Transfus Apher Sci* 2020)
Do you quantify in parallel the previous sample and the new one?	Yes	No

How do I… or How do we…

How do I

	BloodCenter of Brittany, France	Norwegian National Unit for Platelet Immunology
Are there any national official recommendations for quantitation?	No	Yes
Threshold/cut-off to determine severity level	≥28 IU/mL	≥3 IU/mL
How is the results from the quantification used to determine treatment , delivery time/mode and follow up?	Results as used to predict severe fetal /neonatal thrombocytopenia (<50 plt count), and monitoring of pregnancy/delivery: • First trimester, before IvI treatment: when Ab level is high (≥ 28 IU/mL), it is useful to start earlier IvIG • Follow-up during pregnancy (Area Under Curve ≥ 23 IU/mL/WG): - way of delivery (CS) - fresh HPA-1bb platelets	Results are used to advice clinical follow-up of women without history of FNAIT with ICH: ≥3 IU/mL at any time during pregnancy: • Fetal ultrasound every 4 weeks from GW 28 • Referral to hospital with neonatal intensive care unit • Delivery by CS at GW 38-39 • HPA-1bb platelets available (Tiller et al. *Transfus Apher Sci.* 2020;59 (1) 102711)

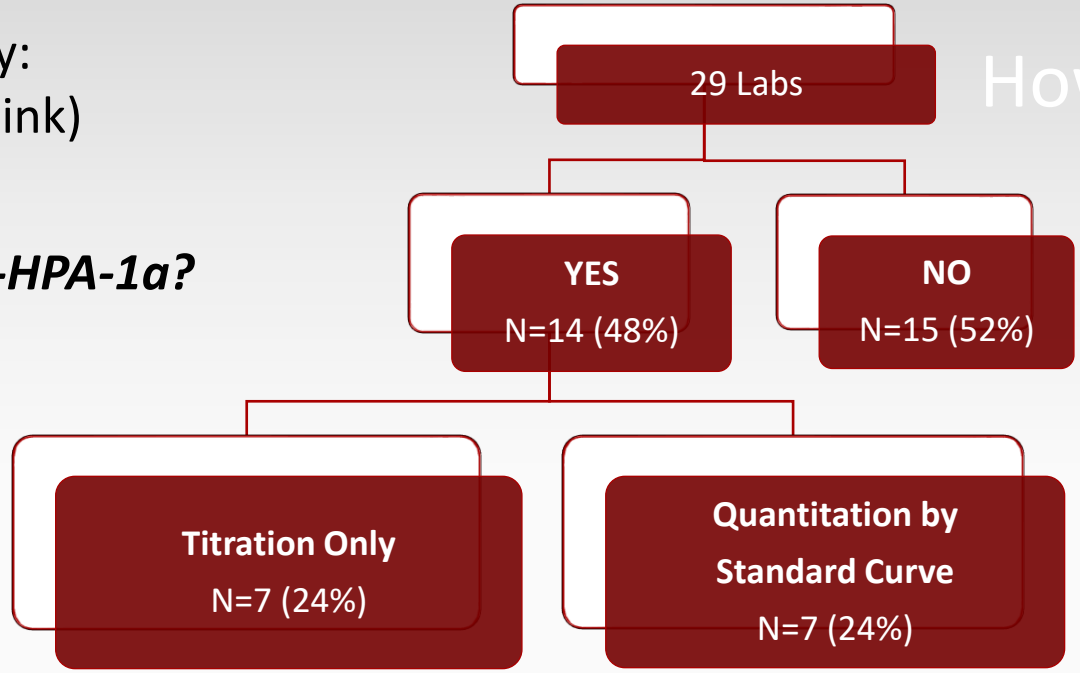
Survey on Quantitative MAIPA by 2018 Working Party

How do I

From this 2018 ISBT 19th Platelet Workshop Survey:
Prepared by Héma-Québec, Canada (reference or link)

Do you quantitate anti-HPA-1a?

How do you do it?



10 out of 29 laboratories provided their quantitative MAIPA protocol for anti-HPA-1a quantification.

The protocols varied in with respect to:

- *Platelet input per well/tube: (10-25 x10^6 platelets)*
- *Mouse monoclonal anti-glycoprotein antibody clones*
- *Reference standard sera: in-house sera, NIBSC minimum potency, NIBSC International standard (100IU/mL) or monoclonals.*
- *Assay substrate: OPD or TMB*

How do I… or How do we…

2018 Working Party: Survey on Quantitative MAIPA, Héma-Québec, Canada

How do I

Why we do it… from the survey over 29 reference laboratories

Question asked:	Answers	Number of labs of 29 participants	% of the 14 labs doing quantification	% of the 29 reference labs
Do you perform anti-HPA-1a antibody quantification:	Yes	**14**	-	**48%**
	No	15		52%
Do you think antibody quantity correlates with the baby's platelet count:	Yes or sometimes	**8**	**57%**	**28%**
	No	21		72%
Do you think antibody quantity correlates with intracranial hemorrhage:	Yes	**8**	**57%**	**28%**
	No	21		72%
Does quantity of Ab dictate your recommendations during pregnancy:	Yes	**9**	**64%**	**31%**
	No	20		69%
Do you report antibody quantity:	Yes always	**4**	**29%**	**14%**
	No or sometimes	25		86%
Does antibody quantification guide the treatments:	Yes	**9**	**64%**	**31%**
	No or don't know	20		69%

2018 Working Party: Survey on Quantitative MAIPA

Héma-Québec, Canada

How do I

Should we do it… Striking Facts…

From the 2018 ISBT 19th Platelet Workshop Survey:

- 14 out of 29 laboratories (48%) mentioned using quantification for at least anti-HPA-1a antibodies.
- 10 labs (34%) submitted a quantitative MAIPA protocol for anti-HPA-1a.
- but only 4 labs (14%) regularly report their quantitative results.
- Only around 64% of the labs performing quantification say that they use it in their recommendations.

No official standardized assay for quantification is available. Everybody seems to adapt and modify the MAIPA assay according to some historical and/or technical limitations.

Standardization of antibody reaction by determining an IU/mL activity is not widely performed through platelet reference labs.

Quantification by comparison to an antibody standard does not necessarily take into account the avidity and affinity of the antibody.

How do I quantify anti-HPA-1a antibodies?

How do I

Some Questions:

- Should every reference laboratory perform anti-HPA-1a antibody quantification?
- Should we consider titration only (without using an official standard) a valuable way of quantification?
- Should we also quantify other anti-HPA antibodies?

How do I quantify anti-HPA-1a antibodies?

How do I

Conlusions:

- Still more questions to be raised and answered…
- We are far away from a standardized quantitative test!
- Quantification is useful when performed by reference labs, where predictive thresholds have been locally defined by cohort studies
- We need more studies and publications to demonstrate the usefulness of anti-HPA-1a antibody quantification and to promote its use among reference laboratories