Next Generation Immunoassay: RAPPID-CompAct

Joanne E. van Keulen[1,2], Marlinne A. M. E. van Roessel[3], Maxime C. M. van den Oetelaar[3], Antonio Ornelas-Soares[3], Maarten Merkx[3,4], Richard B. Pouw[1,2,5].

1. Sanquin Research and Landsteiner Laboratory, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands; 2. Amsterdam Institute for Immunology and Infectious Diseases, Immunology, Amsterdam, The Netherlands; 3. LUMABS BV, Amsterdam, The Netherlands; 4. Laboratory of Protein Engineering, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands; 5. Department of Hematology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.

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Clinical Need

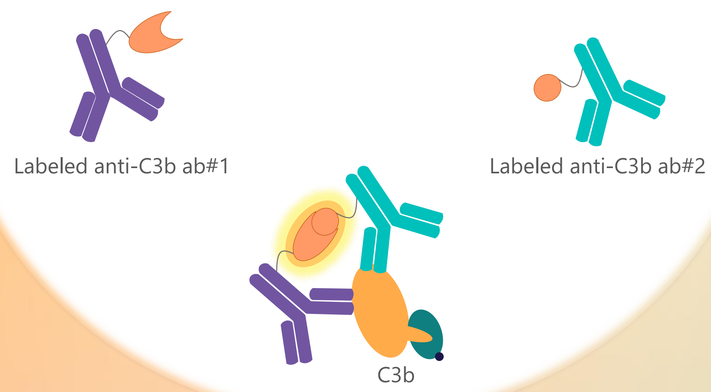
Accurate measurement of aberrant complement activation is critical for diagnosing and therapeutic management of complement-associated diseases.
Problems obtaining reliable complement measurement:

- X Complement activation is short lived *in vivo*
- X Complement activation occurs *ex vivo*
- X Serum proteases degrade complement

Thus, quick measurement is essential.

RAPPID-CompAct Technology

- ✓ Simple sample preparation
- ✓ One-step immunoassay
- ✓ Short analytical assay time



<60 min Protocol

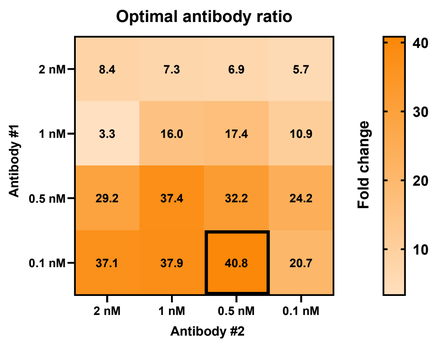
Antibody labeling (5 min)

At room temperature

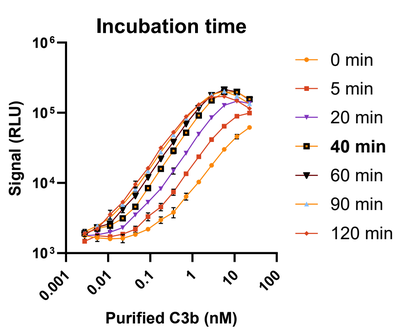
1. Dilution of sample
2. Mixing antibodies, substrate and sample
3. Incubate 5-40 min

Measure with luminometer and analyze

Optimization

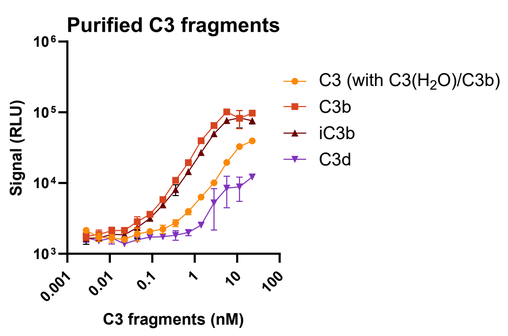


Optimal combination of antibody concentrations was determined based on best C3b to background signal with the lowest antibody concentrations.

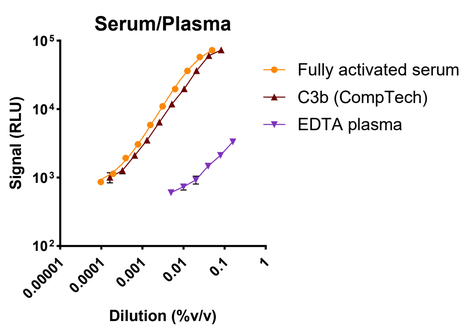


Directly after incubation of the sample with the antibodies and substrate a signal could be obtained. Stable signal was measured after 40 min. Hook effect is present at high C3b concentrations.

Specificity

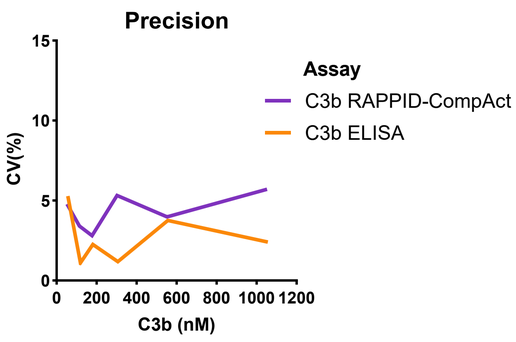


Specificity of the C3b RAPPID-CompAct was tested with purified C3 fragments, showing specificity for C3c. Purified C3 did contain C3(H_2O) and C3b.

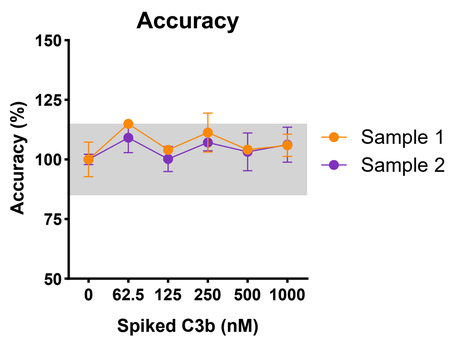


Fully activated C3 in serum gave comparable results as purified C3b. EDTA plasma of healthy donors with low complement activation showed no cross-reactivity with native C3.

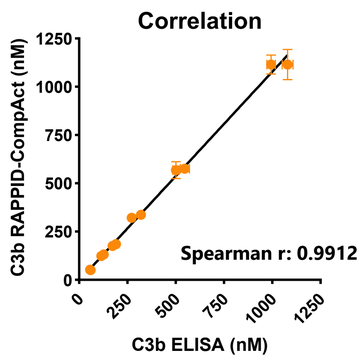
Assay Performance



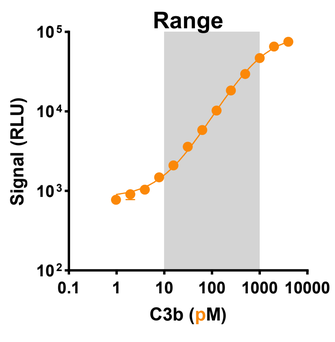
Coefficient of variance (CV) for the C3b RAPPID-CompAct is on average 4.25%.



The accuracy of the C3b RAPPID-CompAct is on average 106.62% (+/- 5.71%). Determined by spiking in C3b from fully activated serum in EDTA plasma of donors.

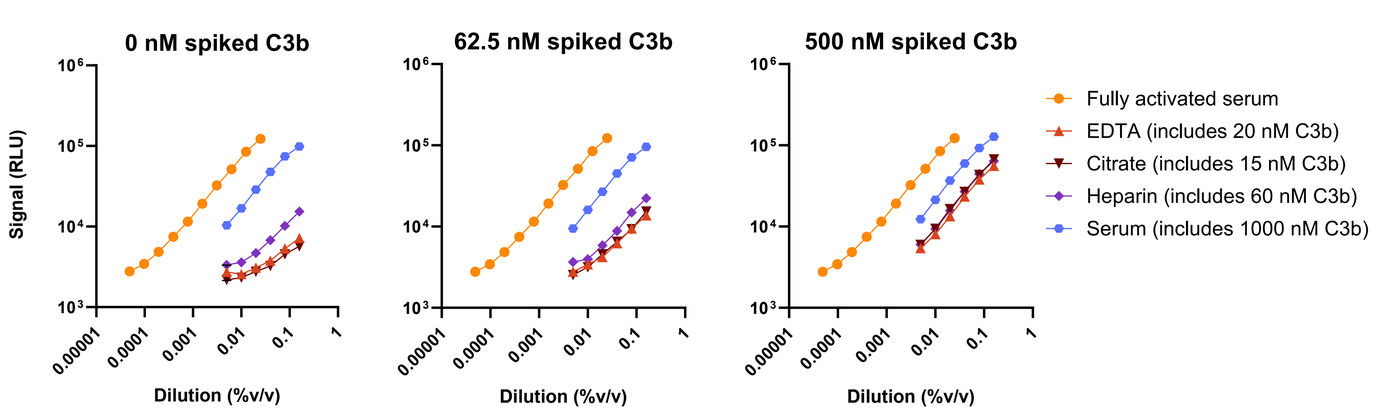


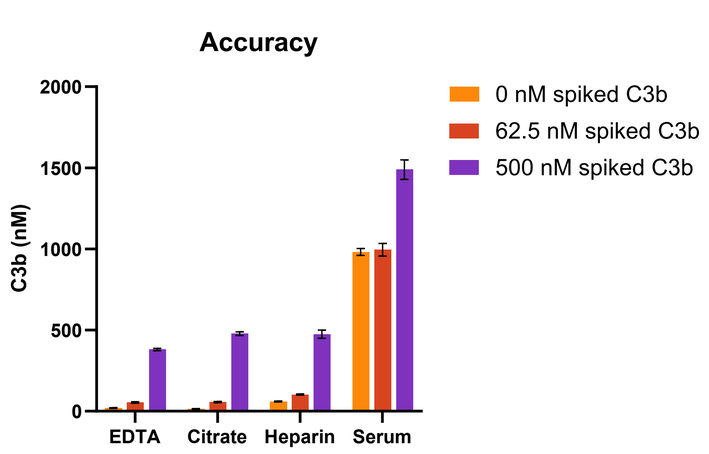
C3b RAPPID-Compact shows a strong correlation with an ELISA containing the same antibody combination.



The C3b RAPPID-Compact is very sensitive. C3b can be detected in the low picomolar range.

Different Matrixes





No interfering effects of different matrixes were observed in the C3b RAPPID CompAct. This was tested by spiking in C3b from fully activated serum into pooled EDTA plasma, citrate plasma, heparin plasma or serum from healthy donors.

Further Optimization

- Validation of C3b RAPPID-CompAct in patient
- Kit industrialization
- Other targets:
  - C3a/C5a, C4b, FH, FHR, Bb, free C5, soluble C5b-9
  - Multiplexing

Applications

- Clinical lab (automated) diagnostics
- Point-of-care
- Clinical trials – patient stratification
- TDM - Monitoring therapeutic efficacy

Interested to try it on your samples/clinical work/clinical trial? Let's talk!

Joanne E. van Keulen
PhD candidate at Sanquin Research, Amsterdam, The Netherlands
Joanne.vankeulen@sanquin.nl

LUMABS BV
www.lumabs.com
Antonio Soares
a.soares@lumabs.com