The Bound-Free Phase Detection Immunoassay (BFPD-IA)
Development, integration, and validation

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Operating principle

BFPD-IA components (indicatively for a **competitive** format): (a) magnetic beads (MB) coupled with capture antibody, (b) fluorescent beads (FB) coupled with competitive antigen, (c) one assay buffer, and (d) the sample.

BFPD-IA steps: (1) Addition of all reagents in one well (75 µL). (2) Single-step incubation. (3) Separation of magnetic beads. (4) Fluorescence detection in the bound-free phase.

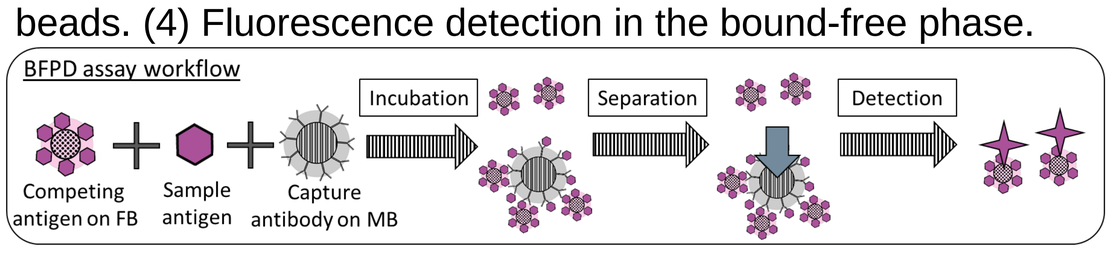


Fig. 1: Workflow of BFPD-IA

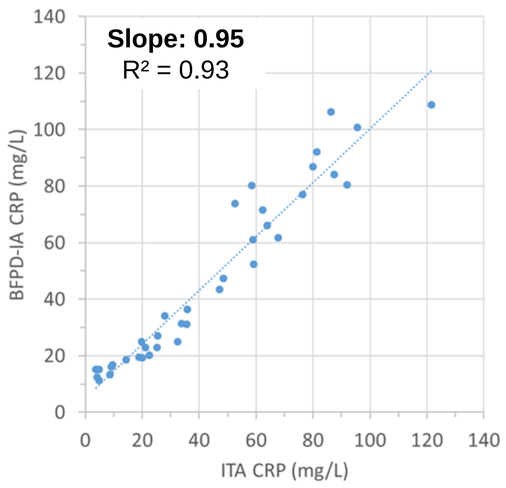
Demonstration in microtiter plate (MTP)[1]

- Diverse **biomarkers** (CRP, MMP-8, MMP-9, TIMP-1)
- Diverse **specimens:** serum, saliva
- **Concentration ranges:** mg/L, ng/mL
- Sandwich and competitive **format**
- Single, du/tri**plex** (fluorescence)
- **Rapid** (~20 min) **wash-free** assay

Validation with clinical samples[2]

- Stratification of patients with Respiratory Tract Infections (RTIs) in concentration zones (low/high cutoffs: 20/100 mg/L)

BFPD-IA vs commercial immunoturbidimetry analyzer



BFPD-IA vs CE-IVD ELISA kit

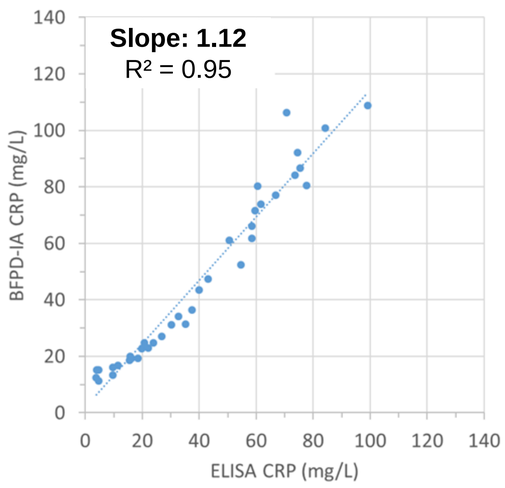


Fig. 2: CRP in clinical serum samples (N = 37)

Integration of BFPD-IA in PCR-performing instruments[3]

- PCR-Disk for pathogen detection
- ImmunoDisk for biomarker detection
- Same instrument for both
- Improved logistics
- Sepsis diagnosis
- Respiratory infections
- Tropical diseases
- Oral diseases

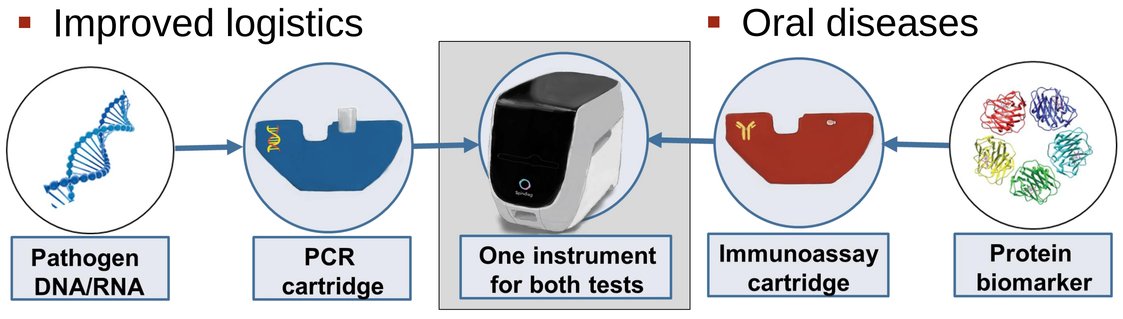


Fig. 3: BFPD-IA enables PCR & Immunoassay on the same instrument

The Sample-to-Answer (S2A) ImmunoDisk[4]

- Workflow: (1) Insert serum. (2) Centrifugally open stickpack & release buffer. (3) Rehydrate fluorescent beads. (4) Rehydrate magnetic beads → incubate → separate → detect all in the same 'multi-purpose' chamber.

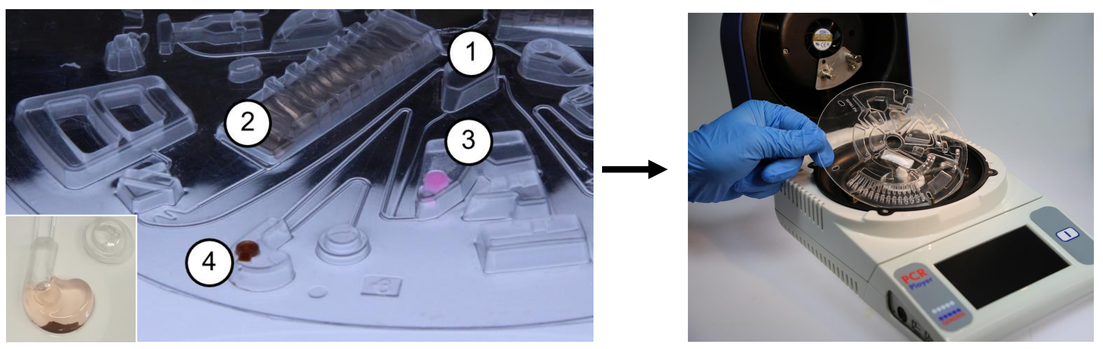


Fig. 4: Left: A sample-to-answer ImmunoDisk, with figure of the separated (centrifuged) MB in the 'multi-purpose' chamber, prior to the FB detection. Right: A functional model of the LabDisk Player, capable of processing Immuno- and PCR-Disks[5].

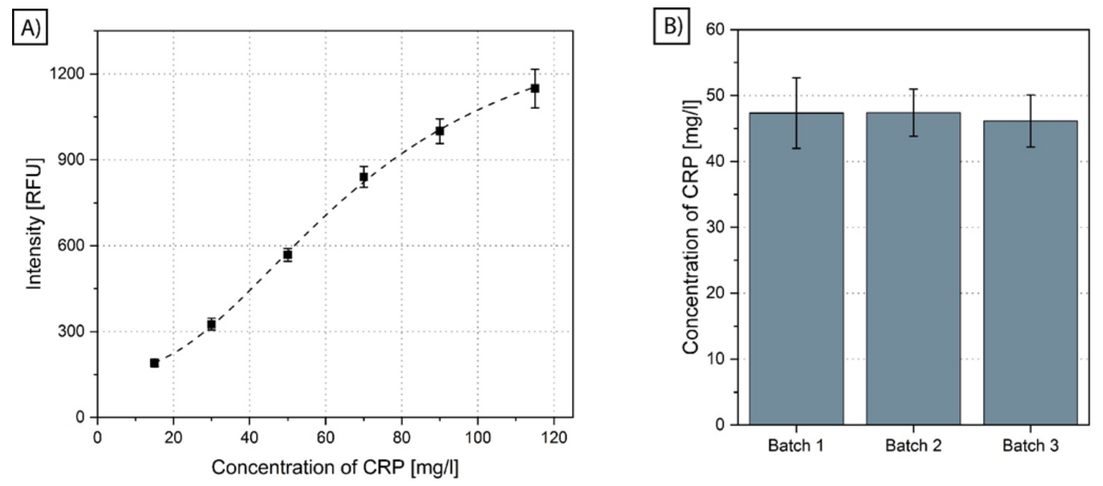


Fig. 5: Left: A standard curve acquired with the ImmunoDisk. Right: CRP Certified Reference Material (CRM) quantified using the ImmunoDisk.

The mid-throughput ImmunoDisk[6]

- 2 half disks per run → 14 data points per run
- 27 clinical samples tested: 81.5% agreement with MTP

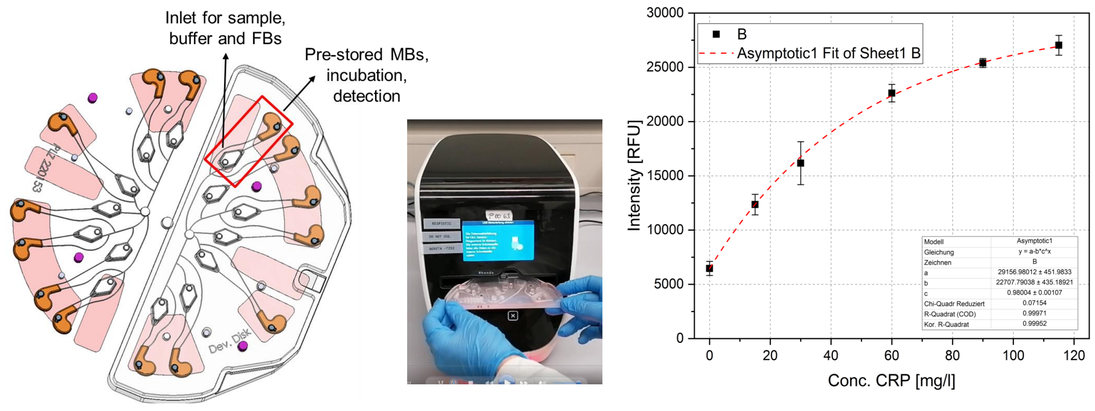
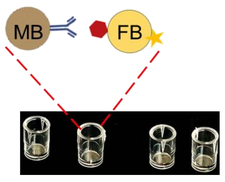


Fig. 6: Left: Design of the mid-throughput ImmunoDisk. Middle: A Rhonda commercial PCR device for which the ImmunoDisk was adapted. Right: A standard curve for measuring 27 clinical samples.

The future



- Integration of BFPD-IA in commercially available reaction wells in non-microfluidic systems
- A robotic liquid handling platform with LAMP isothermal amplification for sepsis (BMBF project DiagnoSeps)[7]
- A compact, battery-operated, LAMP-compatible POC instrument for Respiratory Tract Infections in sub-Saharan Africa (EU project HOLICARE)[8]

[1] B. Johannsen, et al. Anal. Chim. Acta 2021; 1153:338280
[2] B. Johannsen, et al. Biosensors 2023; 13:1009
[3] B. Johannsen, et al. Stud. in Health Technol. Inform. 2020; 273:234-239
[4] B. Johannsen, et al. Biosensors 2022; 12(6):413
[5] M. Rombach, et al. Analyst 2020; 145:7040-7047
[6] B. Johannsen, et al. Proceedings 2024; 97:166
[7] https://www.hahn-schickard.de/en/projects/projects/diagnoseps
[8] https://holicare-project.org/