Supplementary Information

Emerging Multianalyte Biosensors for the Simultaneous Detection of Protein and Nucleic Acid Biomarkers

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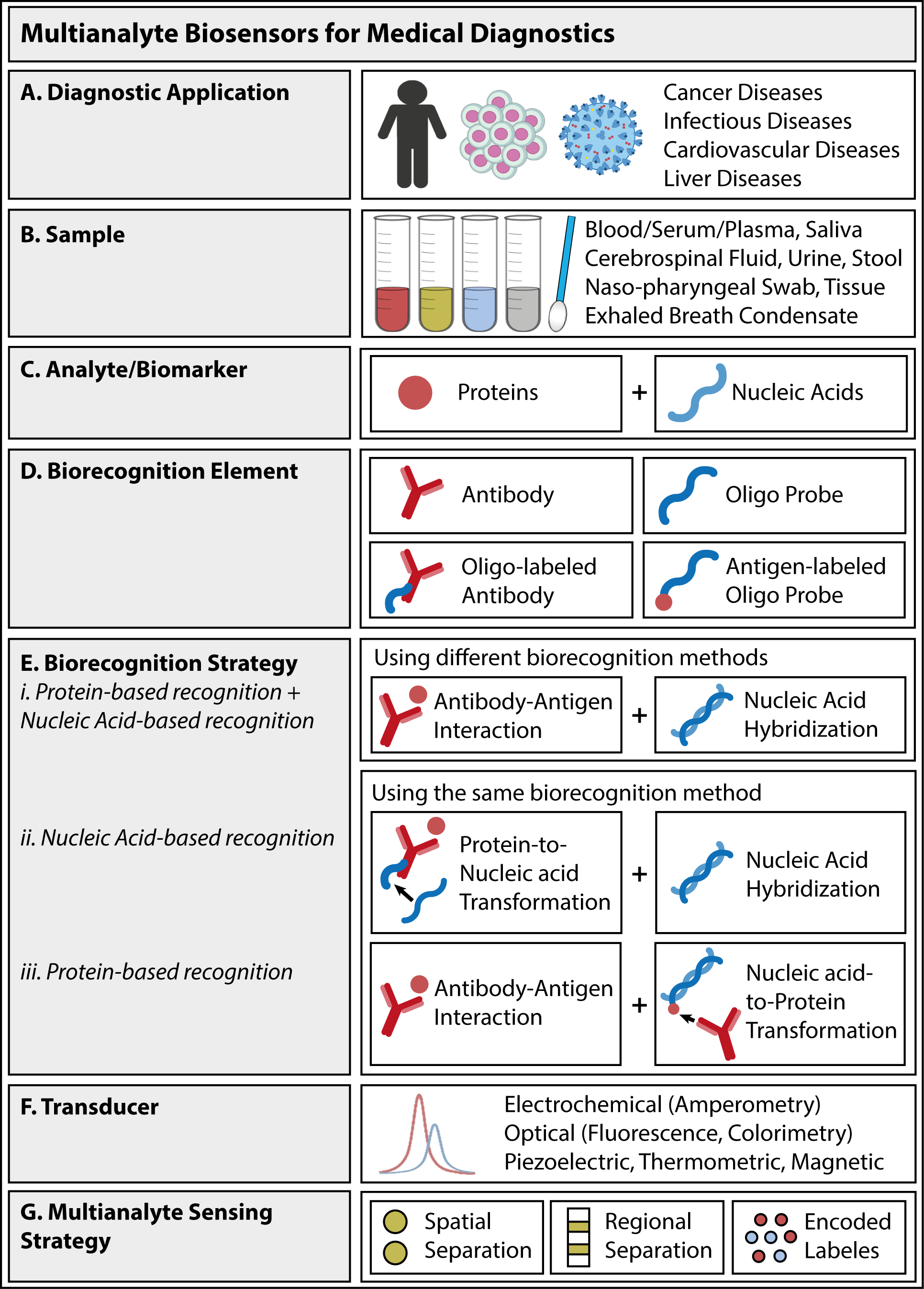
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**Figure S1:** Summary of designing aspects for the development of multianalyte biosensors capable of simultaneous detection of proteins and nucleic acids. **(A)** The diagnostic application defines the requirements (such as sensitivity, selectivity, complexity, costs, and multianalyte sensing capabilities) of the biosensor. **(B)** A suitable sample type (like blood, saliva, or urine) needs to be defined that contains the target biomarkers. **(C)** Development of a comprehensive biomarker panel (including different classes of biomolecules) that can provide more information for an accurate diagnosis and treatment of diseases. **(D)** A suitable biorecognition element needs to be defined that specifically captures the target proteins or nucleic acids. **(E)** Development of a suitable biorecognition strategy that allows the simultaneous detection of proteins and nucleic acids. In this regard, a protein-based recognition can be combined with a nucleic-acid based recognition (i) (using different biorecognition methods) or both biomolecule classes can be detected via nucleic acid-based (ii) or protein-based recognition (iii) (using the same biorecognition method). **(F)** Selection of an appropriate transducer that converts the biorecognition events into a measurable signal. **(G)** Development of a strategy for the simultaneous sensing of multiple analytes to distinguish between signals generated from different biorecognition elements.

**Table S1:** Definition of important terms used in the context of multianalyte biosensors.

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| **Terms** | **Definition** |
| Analyte | A compound or substance (such as inorganic molecules or biomolecules) that is undergoing analysis or is being measured (Harvey). |
| Biomarker | A biomolecule that is found in body fluids or tissue and can be used to follow biological processes and diseases (European Medicines Agency). |
| Biomolecule  (i.e., biological molecule) | An analyte that is produced by cells and living organisms. They perform a wide array of functions and can be classified into four major biomolecule classes – proteins, nucleic acids, carbohydrates, and lipids (European Commission 2007). |
| Biorecognition | Process in which a target analyte binds with high affinity to a biorecognition element (Becherer et al. 2020; Hamzah and Nadzirah 2022). |
| Biorecognition element  (i.e., bioreceptor) | The compound of a biosensor that specifically interacts with a target analyte (Becherer et al. 2020; Hamzah and Nadzirah 2022). |
| Biosensor | A device that uses specific biochemical reactions to detect analytes usually by electrical, thermal, or optical signals (Gold 2019). |
| Biorecognition method | The manner, technique, or procedure by which the presence/abundance of a specific analyte is determined (quantitative or qualitative). For example, the detection of a target protein via an immunoassay (i.e., protein-based recognition). |
| Multianalyte detection | The detection or measurement of more than one analyte from a single sample. This means the simultaneous detection of multiple analytes from the same class and/or multiple analytes from different classes. For example, the simultaneous detection of different proteins, nucleic acids, or the combination of them. |
| Multiomics | The term “omics” implies a comprehensive, or global, assessment of a set of biomolecules (such as proteomics and transcriptomics). Multiomics is the integration of omics data at multiple levels (multiple biomolecule classes) such as genome, epigenome, transcriptome, proteome, and metabolome (Hasin et al. 2017; Subramanian et al. 2020). |
| Technology readiness level (TRL) | A scale that indicates the maturity of a technology (Research Directorate (DRD), Defense Research and Engineering (DDR&E) 2009). |
| Transducer | The transducer is part of the biosensor and converts biorecognition events into a measurable signal (such as electrical, electrochemical, or optical transducers) (Bhalla et al. 2016; Damborský et al. 2016; Hamzah and Nadzirah 2022). |
| Transformation  (i.e., transformative biorecognition) | The process of encoding the abundance of one class of biomolecule into the recognition format of another class of biomolecule. For example, the application of (i) antibodies labeled with nucleic acid oligos for the detection of proteins via nucleic acid-based recognition (protein-to-nucleic acid transformation) or (ii) oligos labeled with antigenic tags for the detection of nucleic acids via protein-based recognition (nucleic acid-to-protein transformation). |

***Table S2: Summary of multianalyte biosensors detecting nucleic acid and protein biomarkers associated with cancer.*** *Multianalyte detection capability: +, low; ++, medium; +++, high; ++++ very high. System complexity: +, low; ++, medium; +++, high; ++++ very high. Time to result: +, < 1 hour; ++, < 4 hours; +++, < 12 hours; ++++, > 12 hours. System integration: +, manual pipetting; ++, some steps are integrated; +++, main steps are integrated; ++++, fully integrated workflow. Ref, Reference.*

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| **Acronym**  **or short description** | **Sample** | **Analyte** | **Biorecognition strategy** | **Multianalyte sensing strategy** | **Transducer** | **Multianalyte sensing capability** | **System complexity** | **Time to result** | **System integration** | **Ref.** |
| Aptamer assisted multiplex-PCR | Plasma, EVs | PD-L1 protein, IDO1 RNA, | NA hybridization +  protein-to-NA transformation | Encoded labels | Fluorescence | + | ++ | ++ | + | (Dong et al. 2020) |
| Biotin-PEG gold nanoparticle-based microarray | Buffer | Prostate-specific antigen and miRNA | Antibody-antigen + NA hybridization | Spatial separation | Light scattering | + | + | ++ | + | (Scott et al. 2017) |
| nCounter® | Tissue | mRNA and proteins such as p35, Met, HER2 | NA hybridization +  protein-to-NA transformation | Encoded labels | Fluorescence | +++ | +++ | ++++ | +++ | (Warren 2018) |
| IMMS | Plasma,  CTC | BRAFV660E protein and DNA | Antibody-antigen + NA hybridization | Spatial separation | Electro-chemical | + | ++ | + | +++ | (Dey et al. 2019) |
| Au NR Dimer-UCNP  Core−Satellite Nanostructures | Cancer cells | Telomerase,  miRNA-21 | NA hybridization +  protein-to-NA transformation | Encoded labels | SERS and luminescence | + | ++ | +++ | + | (Ma et al. 2017) |
| HNCIB | Plasma, EVs | PD-L1 protein and mRNA, miRNA-21 | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | + | ++ | ++ | + | (Zhou et al. 2020) |
| Superwettable microchip | Serum | miRNA-375, miRNA-141, PSA | Antibody-antigen + NA hybridization | Spatial separation | Electro-chemical | + | + | ++ | + | (Xu et al. 2018) |
| DFDT | Serum | MUC1,  miRNA-21 | NA hybridization +  protein-to-NA transformation | Encoded labels | Fluorescence | + | ++ | ++ | + | (Yang et al. 2019) |
| dU-BIO-HP-based assay | Cancer cells | Telomerase protein and RNA | NA hybridization +  protein-to-NA transformation | Encoded labels | Fluorescence | + | ++ | ++ | + | (Yin et al. 2019) |
| Resonance light scattering sensor | Serum | AFP,  miRNA-122 | NA hybridization +  protein-to-NA transformation | Encoded labels | Resonance light scattering | + | + | + | + | (Chen et al. 2018) |
| Dual amperometric magneto-biosensor | Saliva | IL-8 protein and mRNA | Antibody-antigen + NA hybridization | Spatial separation | Electro-chemical | + | + | + | ++ | (Torrente-Rodríguez et al. 2016) |
| RNAscope-based in situ hybridization, antibody staining | Tissue | protein and mRNA: HER2, CK19, CXCL10 | Antibody-antigen + NA hybridization | Encoded labels | Mass-based | +++ | +++ | ++++ | + | (Schulz et al. 2018) |

**Table S3: Summary of multianalyte biosensors detecting nucleic acids and protein biomarkers associated with infectious diseases.** Multianalyte detection capability: +, low; ++, medium; +++, high; ++++ very high. System complexity: +, low; ++, medium; +++, high; ++++ very high. Time to result: +, < 1 hour; ++, < 4 hours; +++, < 12 hours; ++++, > 12 hours. System integration: +, manual pipetting; ++, some steps are integrated; +++, main steps are integrated; ++++, fully integrated workflow. Ref, Reference.

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| **Acronym**  **or short description** | **Sample** | **Analyte** | **Biorecognition strategy** | **Multianalyte sensing** | **Transducer** | **Multianalyte sensing capability** | **System complexity** | **Time to result** | **System integration** | **Ref.** |
| Evalution | Buffer | RSV A&B, TNF-α, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10 | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | +++ | ++ | + | +++ | (Falconnet et al. 2015) |
| Multianalyte LFIA | Buffer | IL-6, *P. aeruginosa* DNA | Antibody-antigen + NA-to-protein transformation | Regional separation | Fluorescence | + | + | + | + | (Klebes et al. 2022) |
| Centrifugal microfluidic platform | Stool | Bacterial toxins, *C.  jejuni*, *E. coli*,  *S. typhimuriu* | Antibody-antigen + NA hybridization | Spatial separation | Fluorescence | ++ | ++ | + | +++ | (Phaneuf et al. 2018) |
| ARROW | Naso-pharyngeal swab | SARS-CoV-2 nucleocapsid antigen and RNA | NA hybridization +  protein-to-NA transformation | Encoded labels | Fluorescence | + | ++ | ++ | + | (Meena et al. 2021) |
| ARROW | Buffer | Zika Virus RNA and NS1 protein | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | + | ++ | ++ | + | (Stambaugh et al. 2018) |
| 3D-printed electrochemical LOC biosensor | Saliva | SARS-CoV-2 RNA and host IgG | Antibody-antigen + NA hybridization | Spatial separation | Electrochemical | + | + | ++ | ++++ | (Najjar et al. 2022) |
| Rheonix CARD® | Saliva | Anti-HIV antibody,  HIV RNA | Antibody-antigen + NA-to-protein transformation | Spatial separation | Infrared | + | ++ | + | ++++ | (Chen et al. 2013) |
| Electrochemical biosensor | Urine | Lactoferrin,  16S rRNA of uropathogens | Antibody-antigen + NA hybridization | Spatial separation | Electrochemical | ++ | ++ | ++ | + | (Mohan et al. 2011) |
| DNA-only multianalyte bioassay | Buffer | Thrombin, RNA | NA hybridization +  protein-to-NA transformation | Encoded labels | Fluorescence | + | + | + | + | (Montserrat Pagès et al. 2021) |
| BiosensorX | Nasal swabs, serum | SARS-CoV-2 RNAs, β-lactam | Antibody-antigen + NA-to-protein transformation | Regional separation | Electrochemical | ++ | + | + | ++ | (Johnston et al. 2022) |

**Table S4:** Summary of multianalyte biosensors detecting nucleic acids and protein biomarkers associated with medical diagnostics (other than cancer and infectious diseases). Multianalyte detection capability: +, low; ++, medium; +++, high; ++++ very high. System complexity: +, low; ++, medium; +++, high; ++++ very high. Time to result: +, < 1 hour; ++, < 4 hours; +++, < 12 hours; ++++, > 12 hours. System integration: +, manual pipetting; ++, some steps are integrated; +++, main steps are integrated; ++++, fully integrated workflow. Ref, Reference.

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| **Acronym**  **or short description** | **Sample** | **Analyte** | **Biorecognition strategy** | **Multianalyte sensing strategy** | **Transducer** | **Multianalyte sensing capability** | **System complexity** | **Time to result** | **System integration** | **Ref.** |
| Simoa® Multi-Analyte Technology | Saliva | Cortisol, interleukin-6 and microRNA 141 | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | + | ++ | + | ++ | (Wang and Walt 2020) |
| Quantum dot-doped nanoparticle counting platform | Artificial cerebrospinal fluid | Amyloid β 1-42, tau protein,  miR-146a, and miR-138 | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | ++ | + | +++ | + | (Wu et al. 2021) |
| Flex.flow-based microbead assay | Serum | CRP, BNP, LDL, cfmDNA | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | ++ | ++ | ++ | +++ | (Dinter et al. 2019) |
| seqCOMBO | Serum | Liver-type arginase 1 and miR-122 | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | + | ++ | ++ | + | (Marín-Romero et al. 2021) |
| Neutralizer displacement assay | Buffer | Thrombin,  *E. coli* DNA, ATP, cocaine | NA hybridization +  protein-to-NA transformation | Spatial separation | Electrochemical | ++ | + | + | + | (Das et al. 2012) |
| Triple-helix DNA-functionalized carbon nanotubes-based LFA | Buffer | Thrombin, tDNA | NA hybridization +  protein-to-NA transformation | Regional separation | Colorimetric | + | + | + | + | (Huang et al. 2020) |
| Beacon-like probes based on self-assembled ssDNA-graphene oxide architecture | Buffer | Thrombin, DNA | NA hybridization +  protein-to-NA transformation | Encoded labels | Fluorescence | + | + | + | + | (Zhang et al. 2011) |

**Table S5:** Summary of platforms using single-cell multiomics for the simultaneous detection of nucleic acid and protein biomarkers. Multianalyte detection capability: +, low; ++, medium; +++, high; ++++ very high. System complexity: +, low; ++, medium; +++, high; ++++ very high. Time to result: +, < 1 hour; ++, < 4 hours; +++, < 12 hours; ++++, > 12 hours. System integration: +, manual pipetting; ++, some steps are integrated; +++, main steps are integrated; ++++, fully integrated workflow. Ref, Reference.

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| **Acronym or short description** | **Sample** | **Analyte** | **Biorecognition strategy** | **Multianalyte sensing strategy** | **Transducer** | **Multianalyte sensing capability** | **System complexity** | **Time to result** | **System integration** | **Ref.** |
| REAP-seq | Peripheral blood mononuclear cells (PBMC), naive CD8+ T cells from blood | mRNA and cell surface proteins such as HLA-DR, CD34, CD123, CD117, CD33, CD3, CD8, CD4 | NA hybridization +  protein-to-NA transformation | Encoded labels | Sequencing | ++++ | +++ | +++ | ++ | (Peterson et al. 2017) |
| CITE-seq | Cord blood mononuclear cells | RNA and cell surface proteins such as CD3e, CD4, CD8a, CD19, CD56, CD16, CD11c, CD14 | NA hybridization +  protein-to-NA transformation | Encoded labels | Sequencing | ++++ | +++ | +++ | ++ | (Stoeckius et al. 2017) |
| RAID | Human keratinocytes | mRNA and intracellular (phospho-) proteins such as EGFR, NOTCH1, JAG1, KLK6, phospho-FAK, phosphor-RPS6 | NA hybridization +  protein-to-NA transformation | Encoded labels | Sequencing | ++++ | +++ | ++++ | ++ | (Gerlach et al. 2019) |
| PLAYR | Jurkat cells, NKL cells, PBMC, mouse embryonic fibroblasts, mouse embryonic stem cells | mRNA and proteins such as. CD45, CD8, HLA-DRA, CD11c, CCL4, IFNG | Antibody-antigen + NA hybridization | Encoded labels | Mass-based or fluorescence | +++ | +++ | +++ | ++ | (Frei et al. 2016) |
| PEA/STA | Human breast adeno-carcinoma cell  line MCF7 cells | mRNA and proteins such as AXIN1, MKI67, MET, CDH1, NP1, APC, IGF1R | NA hybridization +  protein-to-NA transformation | Encoded labels | qPCR and Sequencing | ++++ | +++ | +++ | +++ | (Genshaft et al. 2016) |

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| SPARC | Human  embryonic stem cell line HS181 | mRNA and protein such as SOX2, POU5F1, EPCAM, TP53, CDH1, GLO1, FGF2, TP53 | NA hybridization +  protein-to-NA transformation | Encoded labels | qPCR and Sequencing | ++++ | +++ | +++ | ++ | (Reimegård et al. 2021) |
| Immunostaining / smRNA FISH | Human cervix adeno-carcinoma (HeLa) cells | NF- κB protein,  IL-6 mRNA, MCPIP1 protein and mRNA,  EEF2 mRNA | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | +++ | +++ | ++++ | + | (Kochan et al. 2015) |
| Immunostaining / Branched DNA FISH | HIV, HTLV, HBV, HCV, Zika Virus, and Influenza | RNA/DNA: HIV, HTLV, HBV, HCV, Zika, Influenza; Protein: HCV NS5a, HIV-1 p24; MOV10; Influenza PB1 | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | +++ | +++ | +++ | + | (Puray-Chavez et al. 2017; Shah et al. 2020) |
| Immunostaining / Branched DNA FISH | HeLa cells and hiPSC cells | mRNA and protein such as TFRC, LAMP1, EEA1, VPS35, ACTB, c-JUN | Antibody-antigen + NA hybridization | Encoded labels | Fluorescence | +++ | +++ | +++ | + | (Popovic et al. 2018) |

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