RULES BASED MEDICINE
a Q² Solutions Company

Multiplex Immunoassays for Idiopathic Pulmonary Fibrosis

Introduction

Rules-Based Medicine (RBM), a Q² Solutions Company, is a CLIA-certified, multiplexed immunoassay testing laboratory that helps researchers accelerate their therapeutic drug and diagnostic efforts.

Multiple peer-reviewed publications have consistently demonstrated a set of blood-based protein biomarkers associated with idiopathic pulmonary fibrosis (IPF) disease progression. A set of well-qualified assays suitable for use as prognostic or predictive biomarkers within the context for IPF clinical trials is lacking.

In collaboration with the Prognostic Lung Fibrosis Consortium (PROLIFIC), Rules-Based Medicine developed highly optimized and analytically validated blood-based proteomic assays for IPF on the Luminex® multi-analyte profile (MAP), specifically focusing on the most relevant IPF biomarkers related to epithelial damage, fibrosis, inflammation and thrombosis.

RELEVANT IPF BLOOD-BASED BIOMARKERS ASSOCIATED WITH IPF PATHOPHYSIOLOGY	
Epithelial Damage	CA-125, CYFRA 21-1, SP-D, CA-19-9, KL-6
Fibrosis	MMP-7, Tenascin C
Inflammation	CCL-18, CXCL13, sICAM1
Thrombosis	PAI-1

Utilization in IPF Clinical Research

A deeper understanding of disease pathophysiology through blood-based biomarkers can guide the development of more effective and targeted treatments. Preliminary research with the IPF MAP assays showed observable differences in several biomarkers between IPF and normal human serum.[1]

Representative population distribution of biomarkers among IPF patient and normal serum[1]

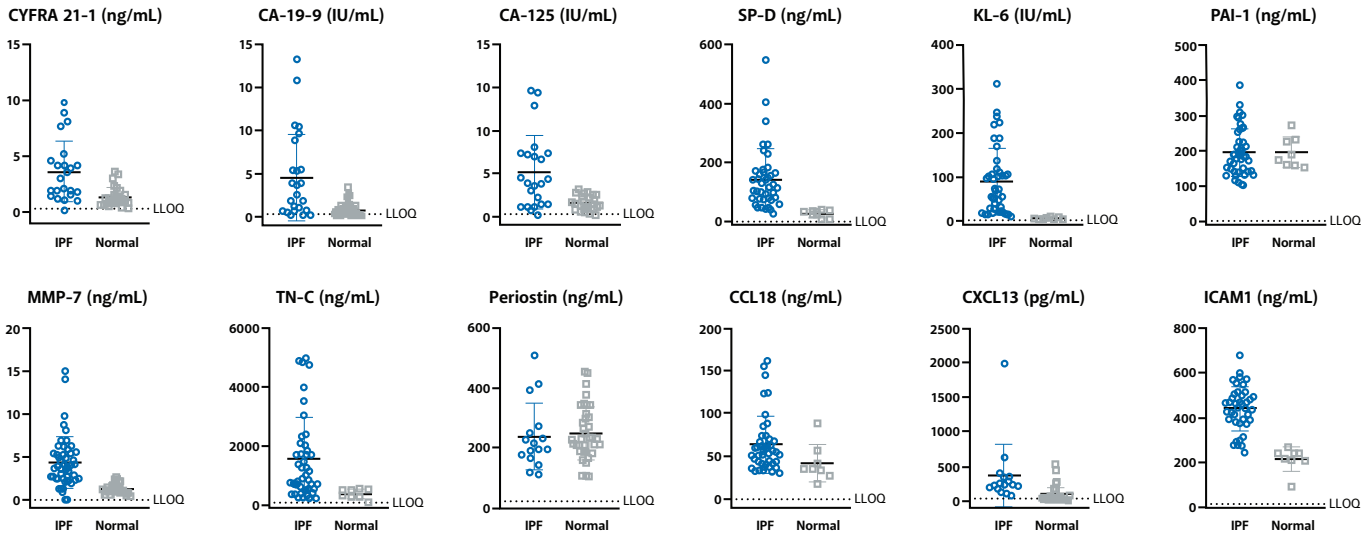

Figure 1. Human serum samples from IPF (n=42, blue) and healthy normal volunteers (n=8, gray). Samples were not matched for age, sex, or BMI. Bar and Whiskers are Mean ± SD.

Utilization in COVID-induced Lung Disease Clinical Research

COVID-19 is associated with long COVID in >10% of patients, often involving the lungs.[2] While COVID-19-associated lung disease and IPF are two distinct diseases, it has been reported that COVID-induced lung disease progresses into a pulmonary fibrosis-like disease[3]; therefore, IPF MAP assays may have clinical utility in this area. Early data supports this concept where eight out of 12 IPF biomarkers were significantly elevated in COVID-19 patient samples compared to normal healthy serum, suggesting a pathophysiological phenomenon similar to IPF that can be further investigated.[1]

Significant elevation of several IPF biomarkers in COVID-19 patient serum vs. normal healthy[1]

CATEGORY	BIOMARKER	HOSPITALIZED COVID-19 (MEAN±SD)	NORMAL CONTROLS (MEAN±SD)	FOLD DIFFERENCE	UNPAIRED T TEST P-VALUE
Epithelial damage	Cytokeratin 19 fragment (CYFRA 21-1), ng/mL	11.5±13.7	1.97±0.966	5.8x	0.0029
	Surfactant Protein-D (SP-D), ng/mL	309±280	59.1±37.2	5.2x	0.0002
	CA-19-9 (sialyl Lewis A), IU/mL	14.7±18.3	9.98±8.14	1.5x	ns
	CA-125 (MUC 16), IU/mL	27.4±26.9	13.6±5.91	2.0x	0.0272
	KL-6 (MUC 1), IU/mL	216±409	10.7±7.9	20.1x	0.0288
Fibrosis	Matrix Metalloproteinase 7 (MMP-7), ng/mL	1.55±0.783	1.58±0.559	0.9x	ns
	Tenascin C (TN-C), ng/mL	3270±3140	711±556	4.6x	0.0006
	Periostin (POSTN), ng/mL	229±173	248±81.9	0.9x	ns
Inflammation	CCL18 (PARC), ng/mL	61.5±38.0	63.7±39.7	0.9x	ns
	CXCL13 (BLC), pg/mL	84.7±47.4	47.2±19.0	1.8x	0.0014
	sICAM-1, ng/mL	423±165	338±101	1.2x	0.0363
Thrombosis	Plasminogen Activator Inhibitor 1 (PAI-1), ng/mL	294±126	210±67.2	1.4x	0.0061

Figure 2. Baseline serum from patients with COVID-19 in clinical trial NCT04472494 (n = 50) vs age-, sex-, and BMI-matched healthy normal volunteers (n = 20). Two-tailed p values from unpaired t test, ns: not significant. One high KL-6 outlier (6390 IU/mL) identified by Grubbs test removed from COVID set.

IPF MAP assays can be utilized as exploratory, prognostic, or predictive indicators in the context of pulmonary fibrosis disease states.

IPF MAP Assays are Highly Optimized and Analytically Validated

The IPF MAP assays were developed and analytically validated with pre-defined acceptance criteria, following the principles of design control. Each lot is manufactured and rigorously qualified for its performance. Analytical validation studies demonstrated high lot-to-lot reproducibility during the qualification of four different lots.

Performance criteria evaluated during the validation process include the lower limit of quantification (LLOQ), the upper limit of quantification (ULOQ), and the dynamic range.

PANEL	ANALYTE	SAMPLE DILUTION	LLOQ	ULOQ	DYNAMIC RANGE	UNITS
IPF1	ICAM-1	1:50	32	17,300	3.4-17,300	ng/ml
	KL-6	1:50	1.7	2,450	0.49-2,450	IU/ml
	PAI-1	1:50	1.5	1,680	0.34-1,680	ng/ml
	PARC	1:50	0.85	1,730	0.34-1,730	ng/ml
	SP-D	1:50	11	15,000	3-14,950	ng/ml
	TN-C	1:50	154	228,000	45-228,000	ng/ml
IPF2	CA-125	1:5	9.7	8,150	1.6-8,150	IU/ml
	CYFRA21-1	1:5	0.51	326	0.065-18,000	ng/ml
IPF3	BLC	1:5	14	18,000	3.6-18,000	pg/ml
	Periostin	1:5	18	18,500	3.7-18,450	ng/ml
IPF4	MMP-7	1:10	0.05	153	0.031-153	ng/ml
IPF5	CA-19-9	1:5	2.1	2,940	0.59-2,940	IU/ml

Table 1. Representative data: The range of each standard used to produce the standard curve multiplied by the dilution factor used for testing human serum or EDTA plasma samples, previously stored at -80°C.

Summary of additional parameters evaluated with defined acceptance criteria met include[4]:

1. **Accuracy of controls:** <20% difference from nominal value for each control level.
2. **Inter-assay precision of quality controls:** <25% for Level 1 and <20% for Levels 2 and 3.
3. **Interference:** None observed for the following interferents tested: bilirubin, hemoglobin, and triglyceride.
4. **Lot-to-lot reproducibility:** slope between 0.9-1.1 for all analytes.
5. **Freeze-thaw (endogenous analyte) stability:** 70-130% recovery for two of three samples across all analytes after five freeze-thaw cycles.
6. **Short-term, 24-hour refrigeration (endogenous analyte) stability:** 70-130% recovery for two of three samples.
7. **Real-time 24-month reagent stability:** Four of six control results within 2.5 SD of the established control values.
8. **Cross-reactivity and specificity:** A 100% specificity was observed in the presence of other IPF immunoassay analytes and ≤0.0% cross-reactivity, except for SP-D (≤0.5%).

For detailed acceptance criteria, view the poster presented at the 7th annual Idiopathic Pulmonary Fibrosis (IPF) Summit in Boston in September 2023.

The RBM MAP platform has been built and optimized to help researchers accelerate their drug and diagnostic efforts with:

High-throughput multiplexing with automated liquid handling to reduce sample volume

Enhanced accuracy with the use of multi-level controls, standard curves, and proprietary blockers

High quality and high reproducibility with stringent quality control parameters for reliable data

With RBM manufacturing the IPF MAP assays and testing your samples, you can ensure quality results for every analyte, every sample, every time.

Idiopathic Pulmonary Fibrosis MAP Assays

The RBM IPF MAP Assays are highly optimized and validated Luminex-based sandwich immunoassays.

IPF1

Intercellular Adhesion Molecule 1
(ICAM-1, CD54)

Krebs von den Lungen-6,
(KL-6)

Plasminogen Activator Inhibitor 1
(PAI-1)

Pulmonary and Activation-Regulated Chemokine
(PARC, CCL18, MIP-4, DC-CK1)

Surfactant protein D
(SP-D)

Tenascin C
(TN-C)

IPF2

Cancer Antigen 125
(CA-125, Mucin 16)

Cytokeratin 19 fragment
(CYFRA 21-1, Keratin 19)

IPF3

B lymphocyte chemoattractant
(BLC, CXCL13, B-cell-attracting
chemokine-1 [BAC-1])

IPF4

Cancer Antigen 19-9
(CA 19-9, sialyl-Lewis)

IPF5

Matrix Metalloproteinase-7
(MMP-7)

References

1. Schafer, P. (2022, August). PROLIFIC Consortium Biomarker Research for PF Clinical Trials. [Scientific presentation]. 6th Annual IPF Summit, Boston, MA.
2. Davis, H. E., McCorkell, L., Vogel, J. M., & Topol, E. J. (2023). Long COVID: major findings, mechanisms and recommendations. *Nature reviews. Microbiology,* 21(3), 133–146. https://doi.org/10.1038/s41579-022-00846-2
3. Justet, A., Zhao, A.Y., & Kaminski, N. (2022). From COVID to fibrosis: lessons from single-cell analyses of the human lung. *Human genomics,* 16(1), 20. https://doi.org/10.1186/s40246-022-00393-0
4. Eisinger, D., Kemp, J., Melin, J., Nitka, K., Bencher, R., Rassa, J., Seyda, A., Hersey, S., and Schafer, P. (2023, September). Development and Validation of Five Idiopathic Pulmonary Fibrosis (IPF) Biomarker Panels Comprising 12 Immunoassays: Advancing Clinical Diagnostic, Pharmacodynamic and Disease Activity Assessments in IPF. [Poster presentation]. 7th Annual IPF Summit, Boston MA.

Contact us

Email: RBM_clientservices@q2labsolutions.com
Website: RBM.Q2LabSolutions.com

RULES BASED MEDICINE
a Q² Solutions Company