# Evaluation of various immunoassay technologies for measuring cytokines in cellular supernatants in a 384-well format

Ziyu Li, Luisa Hofmann, Sonja Mueller, Bodo Brunner

**R&D, Integrated drug discovery, Sanofi** 

All authors are Sanofi employees and may hold shares and/or stock options in the company

## INTRODUCTION

Cytokines play a crucial role as immune mediators, forming the communication network of the immune system. Therefore, understanding the relative balance of cytokines is essential for unraveling biological pathways.

In recent years, a growing array of immunoassay technologies has become available for detecting and quantifying various cytokines. In this study, we present our evaluation data after employing PerkinElmer's HTRF, Promega's Lumit, and Biotechne's Luminex multiplex immunoassays to measure secreted IL-8 and IL-6 cytokines in cellular supernatants using a 384-well format. We will demonstrate the comparison of robustness and sensitivity by calculating the potencies of compounds that inhibit the secretion of IL-6 and IL-8.

# **PRINCIPLE of ASSAY TECHNOLOGY & METHODS**

Secreted human IL-8 or IL-6 in cell supernatant were prepared and measured in a 384-well microtiter plate using three different technologies

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## RESULTS



#### **Standard curves of three detection technologies in 384-well format**



	Dynamic range provided by manufacturers					
	human IL-6 (pg/ml)		human IL-8 (pg/ml)			
	low	high	low	high		
HTRF	51	7500	32	4000		
mmunoassay	18	25000	7.3	10000		
ex multiplex	0.5	370	1.4	1030		

both technologies.

Lumit

Lumin

All three technologies showed linear relationship between provided dynamic range and signal intensities in a 384-well format with reduced assay volume.

Thus, signal intensity is directly proportional to the cytokine concentration.

Signal intensity (raw data) can be directly used to calculate %inhibition or %activation of a compound without the need for conversion into cytokine concentration, making it suitable for high- to medium-throughput testing.

#### **Comparison of HTRF & Lumit immunoassays**

IL-8 measurement: compounds C22, 72 were measured for inhibition of IL-8 secretion in supernatant of a human stable cell line



IL-6 measurement: compounds C23, 57 were measured for inhibition of IL-6 secretion in supernatant of human primary cells



#### **Comparison of HTRF & Luminex multiplex** immunoassays

IL-6 and IL-8 measurement: compound C147 was measured for Inhibition of IL-6 /8 secretion in supernatant of human primary cells



Raw data calculated in %Inhibition

Raw data calculated in %Inhibition

Raw data calculated in %Inhibition

Regardless of the different cytokines as readouts, similar IC50 values were obtained with



## DISCUSSION

- Three immunoassays have proven robustness in a miniaturized 384-well format and can be effectively utilized for medium to high-throughput testing.
- PerkinElmer's HTRF and Promega's Lumit immunoassays offer ease of use, making them optimal for single readout experiments.
- Biotechne's multiplex assay is more complex in terms of sample preparation, but it offers advantages when more analytes (up to 50) are required.
- Based on the listed prices, the HTRF technology costs €1.9 per data point for a 500-assay kit, while the Lumit immunoassay costs €0.99 per data point for an assay in a 384-well plate. For Biotechne's Luminex multiplex assay price is dependent on the number of analytes, e.g. a 100-assay kit with 4-Plex including IL-6 /IL-8 costs €0.6 per analyte in a 384-well plate.

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## Please reach out Ziyu.Li@sanofi.com to get an accessible version of this information.

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HTRF