Thesis Progression

# Quality control and storage effect against anti-IFN-gamma autoantibody quantified by inhibitory ELISA

28th January, 2026

Mr. Truong Hung Cuong - 2nd year Master's Degree Student

Student ID: 675070035-6

Advisor: **Asst.Prof.Dr Arnone Nithichanon**

Department of Microbiology, Faculty of Medicine, Khon Kaen University

# BACKGROUND AND RATIONALE OF RESEARCH



**Adult-onset immunodeficiency** (AOID) is an uncommon condition characterized by the presence of **anti-interferon-γ autoantibodies** or **AIGAs**

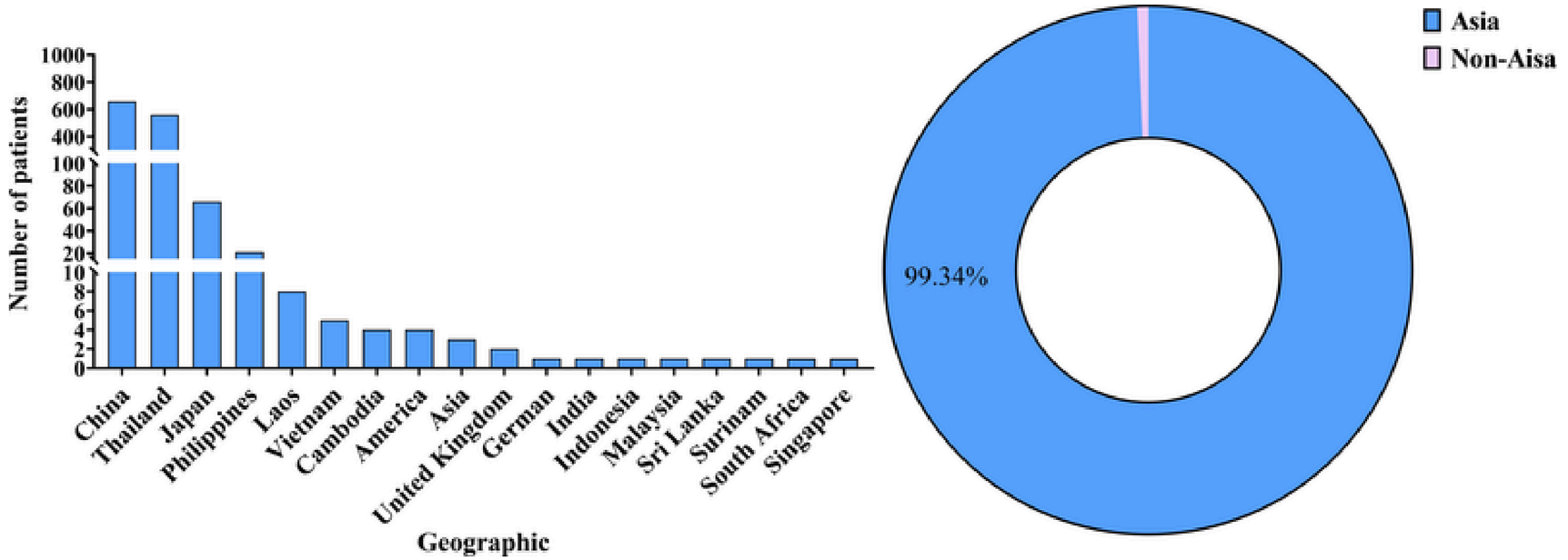



**Figure**. Global AIGAs case distribution (Ni et al., Front. Immunol, 2025)

**Estimated incidence rate of 0.5-1.0 per million people** (Han et al.Current Opinion in Immunology. 2024)

**Death in 11.68% of patients** (Chen N et al. Front. Immunol. 2025)

**Misdiagnosis rate of approximately 33%** (Wu et al. Clin Microbiol Infect. 2020)

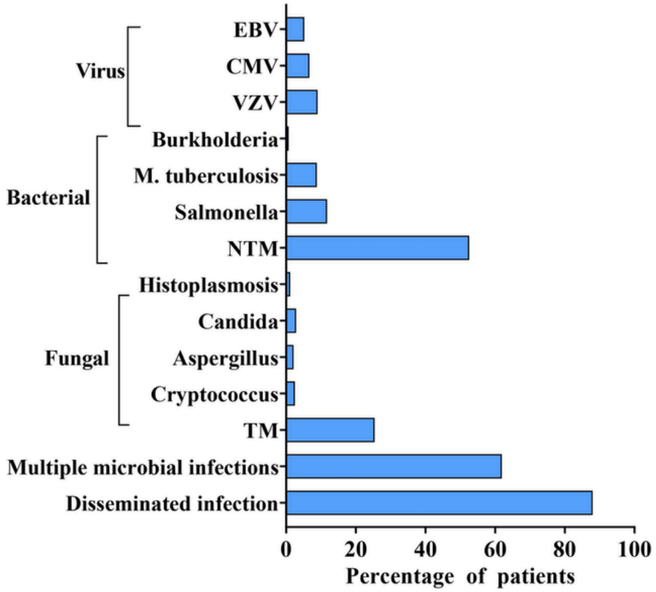
# ADULT-ONSET IMMUNODEFICIENCY SYNDROME (AOID)



## Lymphadenopathy

Karthik et al., Journal of Cancer Research and Therapeutics. 2015

## Simultaneous disseminated infections




(Ni et al., Front. Immunol, 2025)

## Skin lesions

Sangphukieo et al., Genes. 2024

## Clinical misdiagnosed as HIV

# INF-GAMMA AND ANTI-INF-GAMMA AUTOANTIBODIES



- Homodimeric protein, soluble cytokine
- Member of the type II interferon family
- Secreted by NK cells, and specific T cell subsets

## Function

- Activating macrophages
- Suppressing viral replication
- Enhancing MHC class I and II molecule expression
- Amplifying the cytotoxic functions of NK cells and Th1 cells

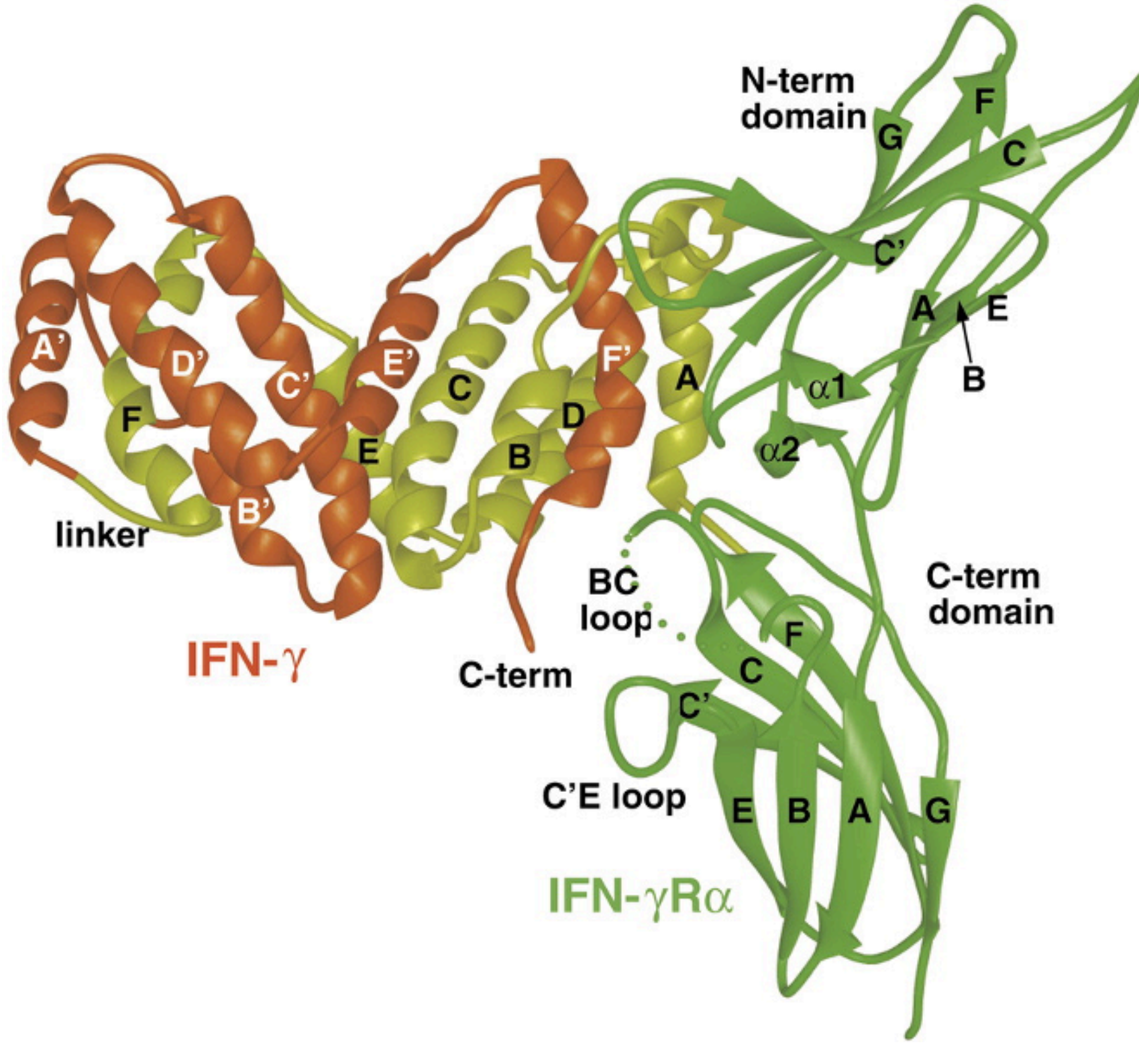



**Figure. Ribbon diagram of the scIFN-γRα 1:1 complex**
(Randal & Kossiakoff, Structure, 2001)

# INF-GAMMA AND ANTI-INF-GAMMA AUTOANTIBODIES



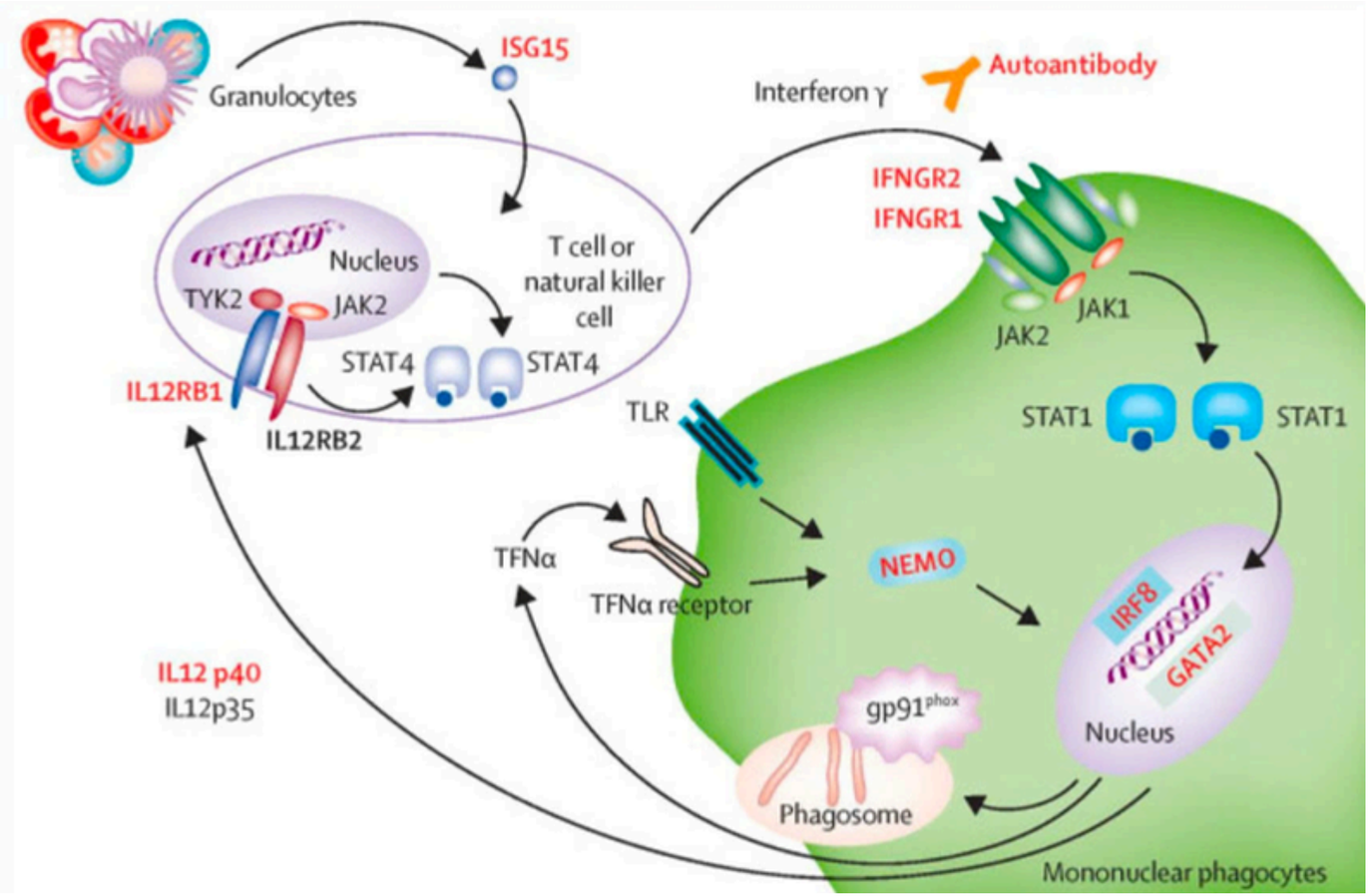



**Figure. Host defense mechanisms against NTM**
(Sharma et al,. Indian Journal of Medical Research. 2020)

- AIGAs are IgG antibodies
- Produced 18 months before appearing clinical signs and symptoms in patients
- Most of the AIGAs target a linear epitope located on the C-terminal tail of the IFN-γ molecule

# DETECTION APPROACHES FOR AIGAS



| | Flow cytometry | Indirect ELISA | Immuno -chromatographic | Inhibitory ELISA |
|---|---|---|---|---|
| **Advantages** | - **Quantitative** measurement based on signal intensity<br>- Can analyze multiple parameters simultaneously | - Easy to perform<br>**- Quantitative**<br>- Standardizable and suitable for batch testing | - Fast (10–30 minutes)<br>**- Convenient, no specialized equipment needed**<br>- Suitable for screening | - Very sensitive for functionally neutralizing antibodies<br>- Suitable for functional studies<br>**- Specificity than Indirect ELISA** |
| **Disadvantages** | - Requires **expensive**, specialized equipment<br>- May be influenced by factors other than the specific autoantibodies<br>**- Find IFN-γ only, cannot quantify AGAIs** | **- False positive**<br>- Requires careful washing to reduce background<br>- Longer assay time than rapid tests | **- Qualitative only**<br>- Lower sensitivity than ELISA | - More complex than indirect ELISA<br>**- Semi-quantitative**<br>- Longer assay time than rapid tests |

# GAP OF RESEARCH



| | Flow cytometry | Indirect ELISA | Immuno -chromatographic | Inhibitory ELISA |
|---|---|---|---|---|
| | - **Quantitative** measurement based on signal intensity<br>- Can analyze multiple | - Easy to perform<br>**- Quantitative**<br>- Standardizable and | - Fast (10–30 minutes)<br>**- Convenient, no specialized equipment** | - Very sensitive for functionally neutralizing antibodies |
| **Disadvantages** | specialized equipment<br>- May be influenced by factors other than the specific autoantibodies<br>- Find IFN-γ only, cannot quantify AGAIs | - Requires careful washing to reduce background<br>- Longer assay time than rapid tests | - Lower sensitivity than ELISA | ELISA<br>- Semi-quantitative<br>- Longer assay time than rapid tests |

- **Lack of acuracy quantitative assay for measuring the anti-IFN-gamma autoantibodies**
- **No database of the quality of the sample affect to the anti-IFN-gamma autoantibodies results**

# RESEARCH QUESTIONS



1. Can the AIGA level be accurately quantified using ELISA, rather than semi-quantitatively measuring AIGA titer?

2. How can a quantitative inhibitory ELISA be standardized through defined steps and key parameters to ensure consistent and reproducible measurement of AIGA levels?

3. How does sample quality, including storage time and temperature affect the accuracy and reliability of quantitative inhibitory ELISA for AIGA quantification?

# OBJECTIVES AND HYPOTHESES



## Objectives

1. To establish a quantitative ELISA that can provide an accurate measurement of AIGA levels from plasma samples
2. To standardize a quantitative inhibitory ELISA for AIGAs detection and ensure its consistent, reproducible quantification
3. To investigate the impact of sample quality, specifically storage time and temperature

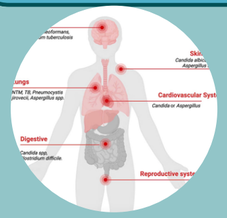
## Hypotheses

1. The quantitative inhibitory ELISA can provide accurate measurement of AIGA levels from plasma samples
2. Systematic standardization of the quantitative inhibitory ELISA for AIGAs detection
3. Storage time and temperature, significantly affects the accuracy and reliability of AIGA quantification

# CONCEPTUAL FRAMEWORK



## PROBLEMS




**Clinical misdiagnosed as HIV**

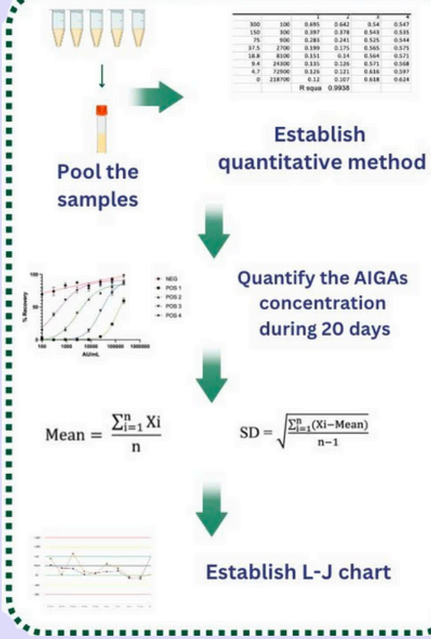
**AOID caused by anti-IFN-γ autoantibodies (AIGAs)**

**Lack of an accurate quantification method for AIGA**

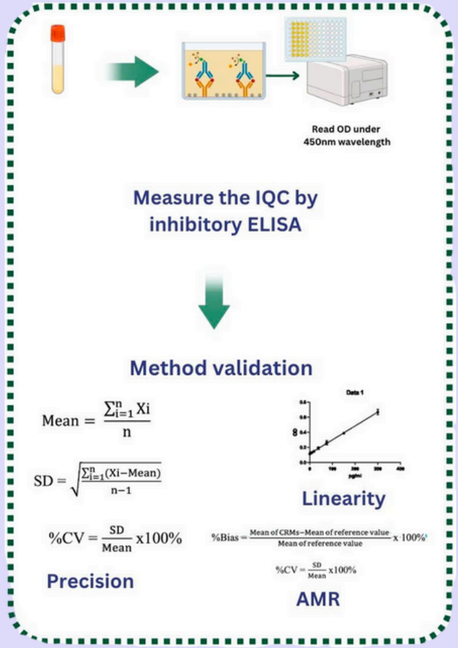
**Impact of storage on sample unclear**

## PROCESS

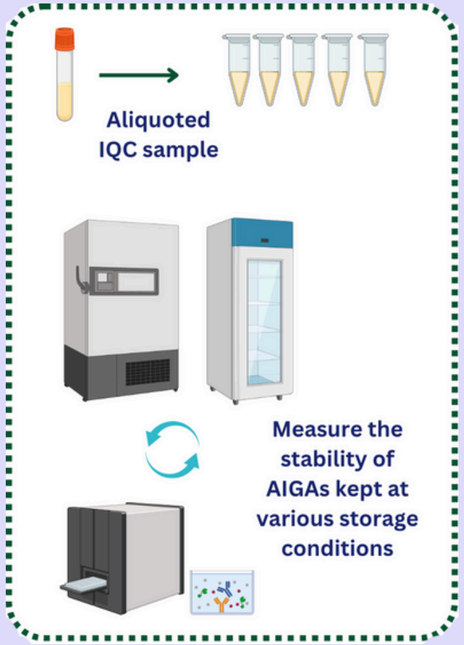
### IQC Sample design




### Standard the method

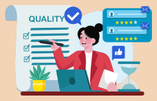



### Storage effect




## OUTPUTS


**Standardized procedure**




**Data of storage effect**

## OUTCOMES


**Standardize Quantitative assay**

**Proper protocol**

## IMPACTS

**Improve clinical making decision**

**Improve dianogstic tool**

# OBJECTIVES AND HYPOTHESES

| Objectives | Hypotheses |
| --- | --- |
| 1 To **establish a quantitative ELISA** that can provide an accurate measurement of AIGA levels from plasma samples | 1 The quantitative inhibitory ELISA can provide accurate measurement of AIGA levels from plasma samples |
| 2 To **standardize** a quantitative inhibitory ELISA for AIGAs detection and ensure its consistent, reproducible quantification | 2 Systematic standardization of the quantitative inhibitory ELISA for AIGAs detection |
| 3 To investigate the impact of sample quality, specifically storage time and temperature | 3 Storage time and temperature, significantly affects the accuracy and reliability of AIGA quantification |

# ESTABLISH QUANTITATIVE ELISA



1. OPTIMIZE OF IFN-GAMMA STANDARD CURVE
2. CREATE INTERNAL QUALITY CONTROL (IQC) SAMPLES
3. SET UP CRITERIA FOR IQC DATA

# OPTIMIZE THE IFN-GAMMA STANDARD CURVE



IFN-standard 600 ng/ml

↓

Perform 2 fold serial dilution in duplicate

↓

ELISA approach

↓

Linear regression

↓

Illustrated by the 3 ranges of linear curve

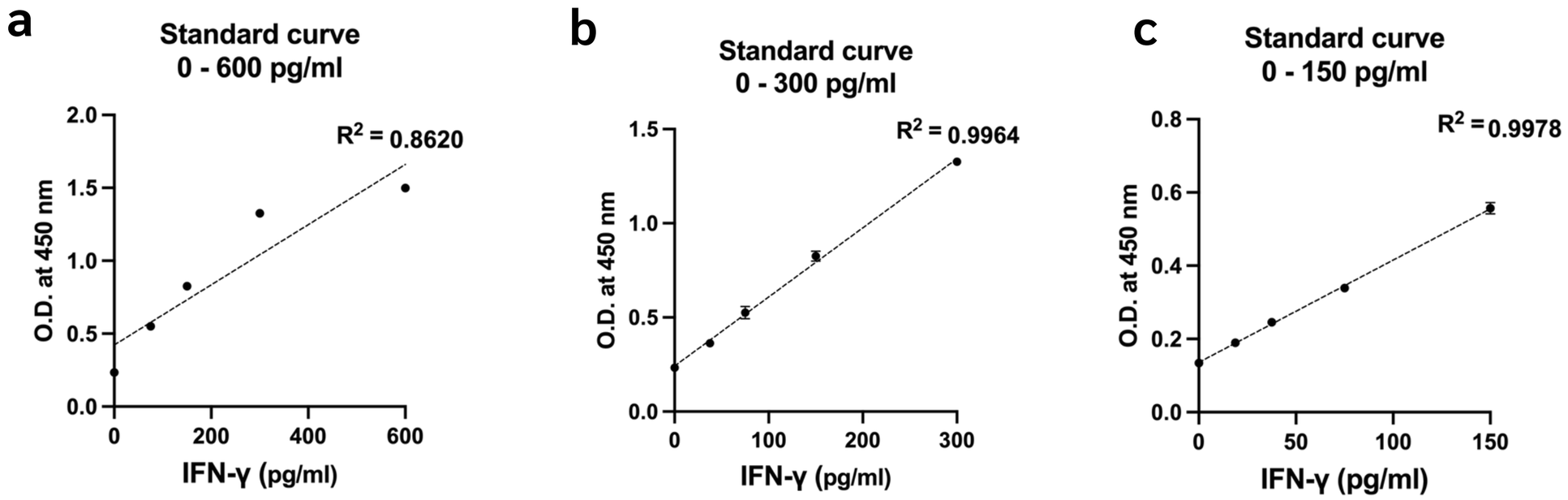



Figure. R-squared of the 3 ranges of IFN gamma standard

The 0–300 pg/mL range was chosen for its good linearity and practical applicability

# OPTIMIZE THE IFN-GAMMA STANDARD CURVE



IFN-standard 300 ng/ml

↓

Perform 2 fold serial dilution in duplicate

↓

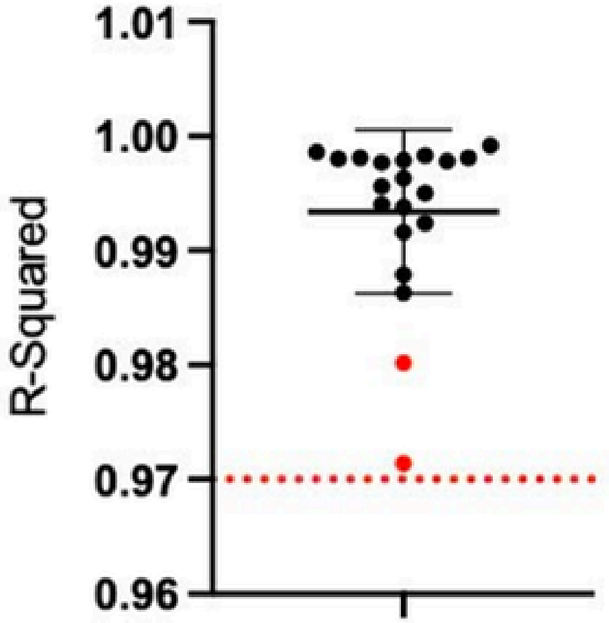
ELISA approach

↓

R-squared calculation

↓

Perform 20 experiments




| | 20 independent experiments |
|---|---|
| Minimum | 0.9714 |
| Maximum | 0.9992 |
| Range | 0.02780 |
| | |
| Mean | 0.9934 |
| Std. Deviation | 0.007136 |
| Std. Error of Mean | 0.001596 |

Figure. R-squared by 20 independent experiments

The acceptance criterion for the standard curve is an $R^2$ value ≥ 0.97

# ESTABLISH QUANTITATIVE ELISA



1. OPTIMIZE OF IFN-GAMMA STANDARD CURVE
2. **CREATE INTERNAL QUALITY CONTROL (IQC) SAMPLES**
3. SET UP CRITERIA FOR IQC DATA

# SAMPLE CRITERIAS



## Healthy Control

1. No history of NTM/Systemic infections
2. HIV-negative status
3. No known immunodeficiency
4. Don't have immunosuppressive medications
5. No acute infection at the time of sampling

## AOID Sample

### Inclusion

Positive blood culture for NTM /Detection of NTM in more than one organ + concurrent /subsequent opportunistic infections

### Exclusion

1. NTM infection limited to the lungs
2. HIV-positive status
3. Nosocomial infections

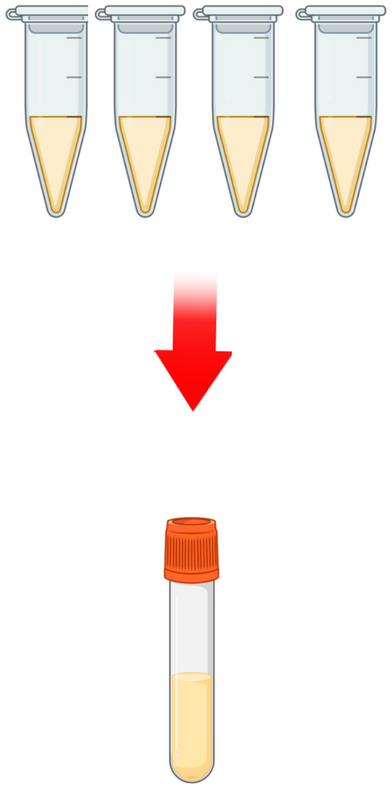
# CREATE IQC SAMPLES



## Negative control

**Healthy control**

AGAIs undetecable result by inhibitory ELISA



## Positive control

**AOID samples**

### Low titre

AGAIs titre by inhibotory ELISA < 100

### Medium titre

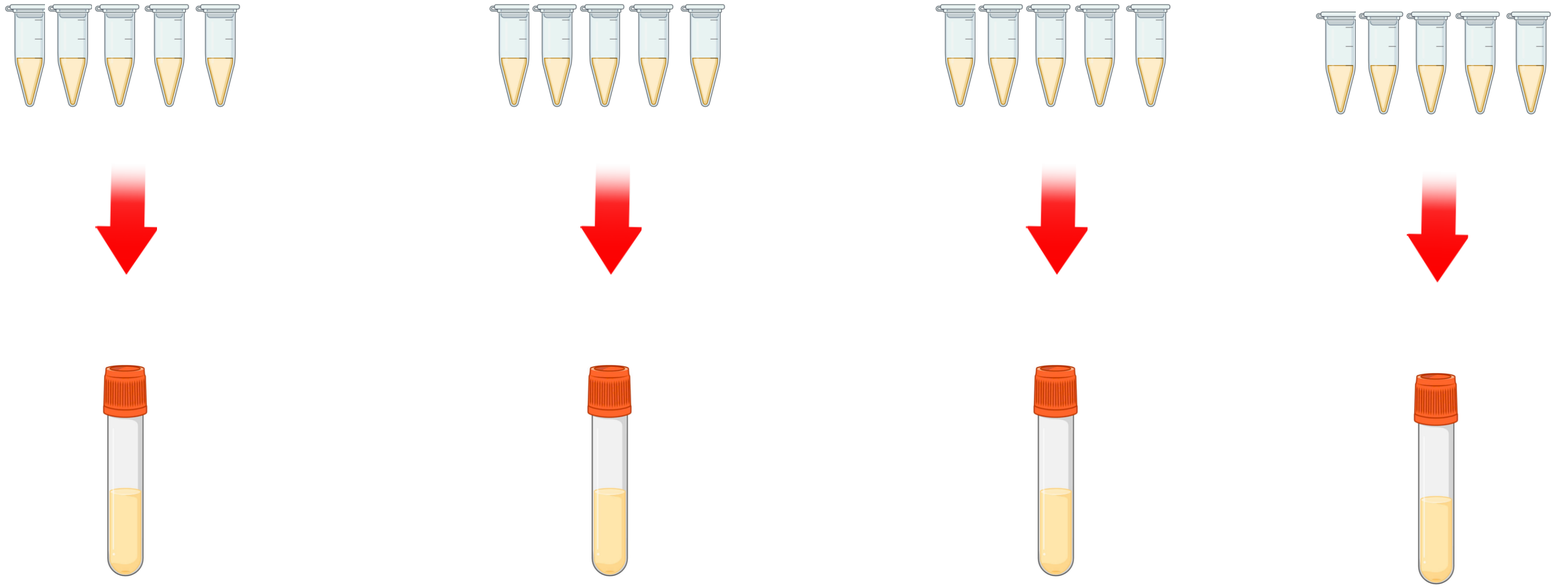
AGAIs titre by inhibotory ELISA 100-1000

### High titre

AGAIs titre by inhibotory ELISA 5000-10000

### Very high titre

AGAIs titre by inhibotory ELISA 10000-100000



# CREATE IQC SAMPLES



IQC sample

↓

Perform 3 fold - serial dilution in duplicate

↓

ELISA aprroach

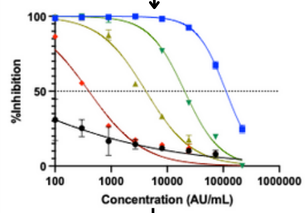
↓

Normalize OD

↓

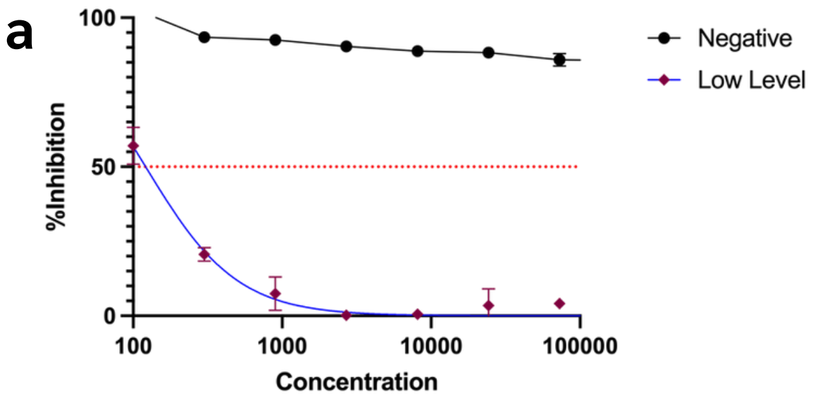
Non-Linear regression

↓



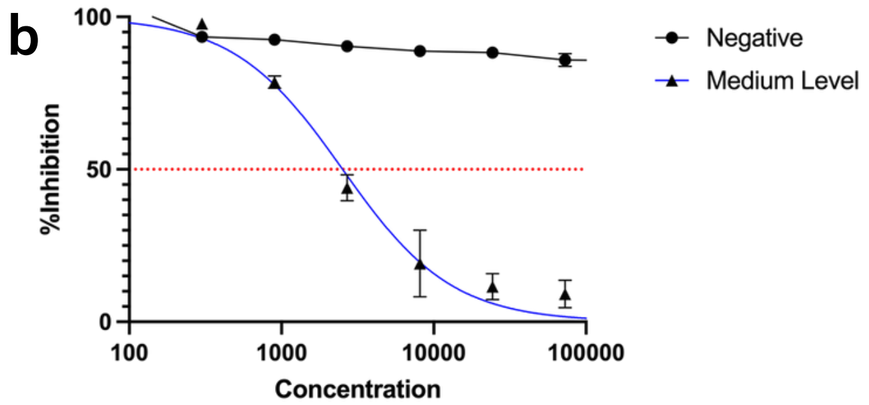

↓

IC50



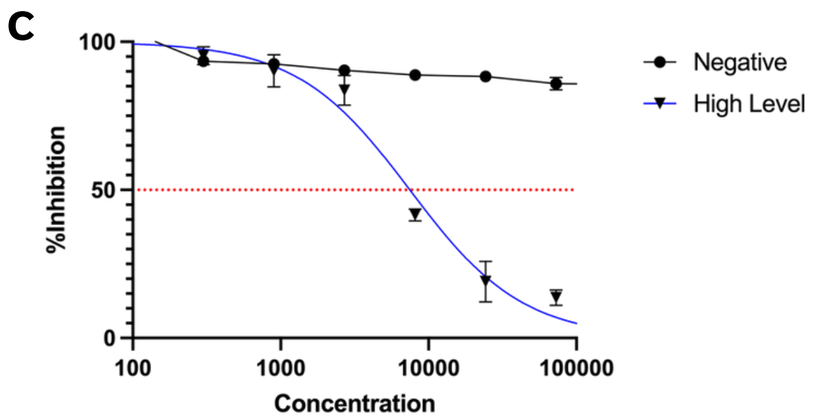

IC50= 263 ($r^2$=0.98)

Low level



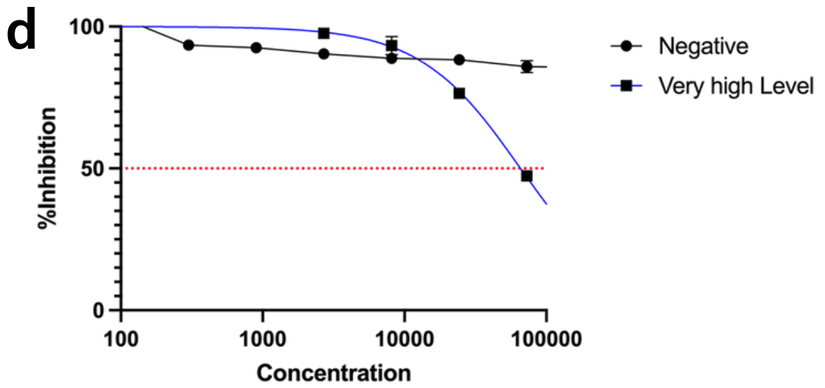

IC50= 4748 ($R^2$=0.98)

Medium level




IC50= 22347 ($R^2$=0.98)

High level




IC50= 128570 ($R^2$=0.97)

Very high level

Figure. R-squared of the IQC samples in independent experiment

# CREATE IQC SAMPLES



IQC sample

↓

Perform 3 fold serial dilution in duplicate

↓

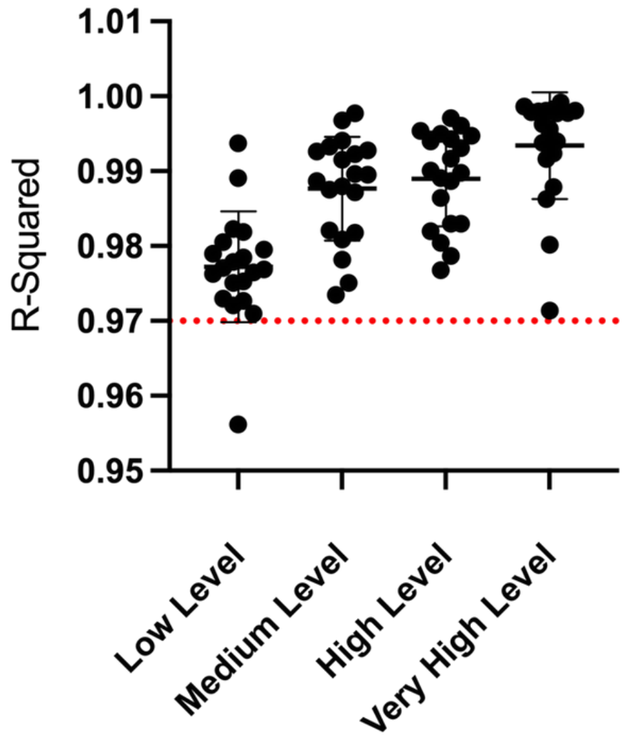
ELISA approach

↓

R-squared calculation

↓

Perform 20 experiments




| R-squared | Mean -3SD | Mean +3SD |
|---|---|---|
| Low level | 0.955 | 0.999 |
| Medium Level | 0.967 | 1.008 |
| High Level | 0.97 | 1.008 |
| Very high level | 0.972 | 1.015 |

Figure. R-squared of the IQC samples during 20 independent experiments

The acceptance criterion for the IQC sample is an $R^2$ value ≥ 0.95

# ESTABLISH QUANTITATIVE ELISA



1. OPTIMIZE OF IFN-GAMMA STANDARD CURVE
2. CREATE INTERNAL QUALITY CONTROL (IQC) SAMPLES
3. SET UP CRITERIA FOR IQC DATA

# SET UP CRITERIA FOR IQC DATA

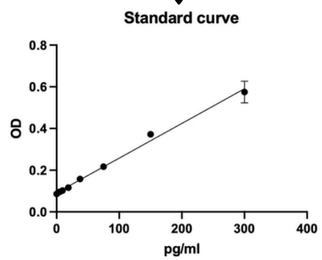


Absorbance at 450 nm

IFN-γ standard

Perform 2 fold serial dilution in duplicate

Linear regression




$R^2 < 0.97$

Reject

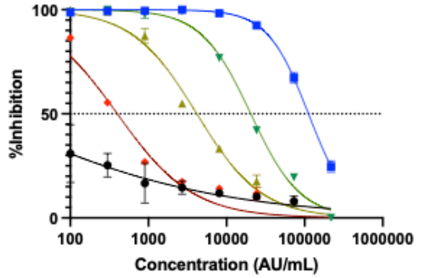
IQC sample

Perform 3 fold serial dilution in duplicate

Normalize OD

$R^2 \geq 0.97$

Non-Linear regression




$R^2 < 0.95$

$R^2 \geq 0.95$

IC50

# OBJECTIVES AND HYPOTHESES

| Objectives | Hypotheses |
| --- | --- |
| 1 To establish a quantitative ELISA that can provide an accurate measurement of AIGA levels from plasma samples | 1 The quantitative inhibitory ELISA can provide accurate measurement of AIGA levels from plasma samples |
| 2 To **standardize a quantitative inhibitory** ELISA for AIGAs detection and ensure its consistent, reproducible quantification | 2 Systematic standardization of the quantitative inhibitory ELISA for AIGAs detection |
| 3 To investigate the impact of sample quality, specifically storage time and temperature | 3 Storage time and temperature, significantly affects the accuracy and reliability of AIGA quantification |

# STANDARDIZE A QUANTITATIVE INHIBITORY ASSAY

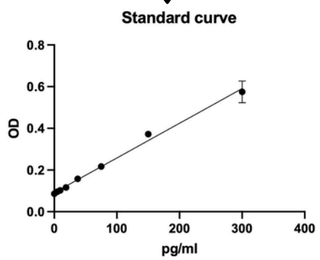


Absorbance at 450 nm

IFN-γ standard

Perform 2 fold serial dilution in duplicate

Linear regression




$R^2 < 0.95$

Reject

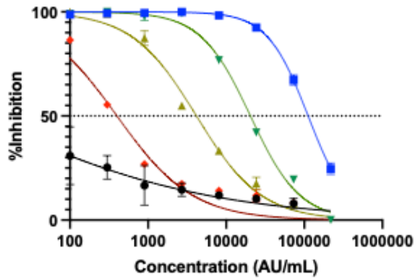
IQC sample

Perform 3 fold serial dilution in duplicate

Normalize OD

$R^2 \geq 0.95$

Non-Linear regression




$R^2 < 0.97$

$R^2 \geq 0.97$

IC50

Perform 20 experiments

Standarize method

# RESULT: LEVEY-JENNINGS CHARTS



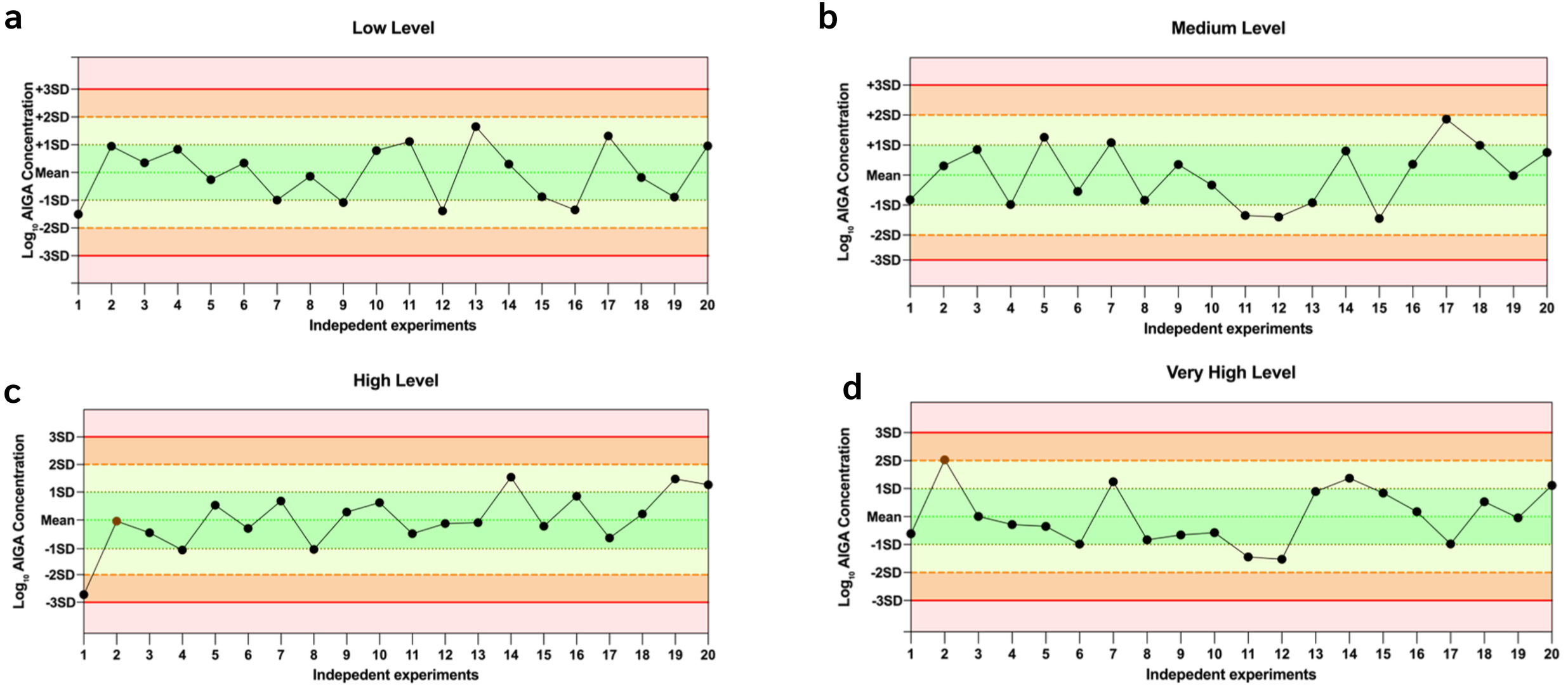



Figure. AIGA concentration during 20 independent experiments illustarted by Levey-Jennings chart

# RESULT: UNITS USED FOR AIGA REPORTING



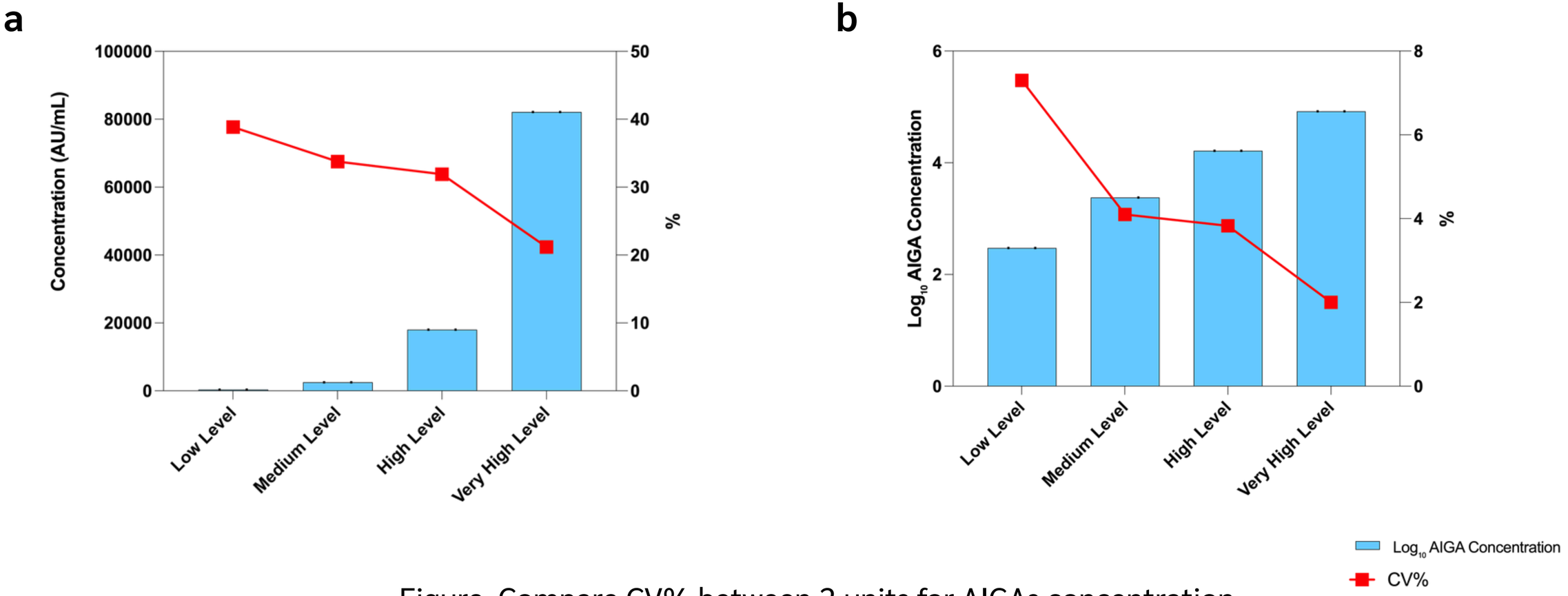



Figure. Compare CV% between 2 units for AIGAs concentration

- The $log_{10}$ concentration unit is appropriate for AIGA reporting
- Low-concentration level is unsuitable for IQC development

# CONCLUSIONS



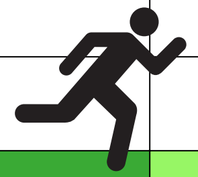
The IFN-γ standard curve shows high linearity across 0–300 pg/mL, supporting the use of an $R^2 > 0.97$ as the criterion for IFN-γ quantification

The AIGAs non-linear regression curves support an $R^2 > 0.95$ as the criterion for IFN-γ quantification

The medium-level IQC sample is optimal for IQC preparation, as it is practical and demonstrates high $R^2$ values and low %CV during long-term repeated measurements

# TIMELINE OF STUDY

| | 2024 | 2025 | | | | 2026 | |
|---|---|---|---|---|---|---|---|
| | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 |
| 1.Literature Review | | | | | | | |
| 2. Practice ELISA assay/ Writing SOP | | | | | | | |
| 3. Creating IQC | | | | | | | |
| 4. Validation method | | | | | | | |
| 5. Measure stability of AIGAs in differ Temp | | | | | | | |
| 6. Measure stability of AIGAs in differ Time | | | | | | | |
| 7. Analyse data | | | | | | | |
| 8. Proposal Examination | | | | | | | |
| 9. Manuscrip Preparation | | | | | | | |
| 10. Thesis Defense Examination | | | | | | | |
| | DONE | | PENDING | | FURTHUR | | |



# ACKNOWLEDGEMENT

Khon Kaen University
Scholarship for
ASEAN & GMS
Countries' Personnel

Medical Microbiology

**Asst.Prof.Dr Arnone Nithichanon**

*Thank You*