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임상시험검체분석기관

백신안전기술지원센터

임상시험검체분석기관 백신안전기술지원센터

(재)백신안전기술지원센터는 약사법 제90조의2에 따라 설립된 식품의약품안전처 산하 비영리 재단법인입니다. 우리 센터는 임상시험검체분석, 백신 품질검사 및 제품화 기술지원을 통해 국내 백신의 신속한 개발을 지원하고자 설립되었습니다.

백신안전기술지원센터 임상시험검체분석기관은 식품의약품안전처로부터 지정('23. 5. 8. 제207 호) 받은 기관으로 GCLP 가이드라인에 따라 전문인력과 시설을 갖추어 면역원성 분석을 실시하고 있습니다. 백신 등 바이오의약품 및 백신 개발을 위한 임상시험검체분석에 백신안전기술지원센터 임상시험검체분석기관이 함께하겠습니다.



백신 등 바이오의약품

- 총 항체가(ELISA 등)
- 중화항체가(PRNT, FRNT, PBNA 등)
- Cytokine 분석(ELISpot)
- Pseudo virus 제작
- 기타 분석 : SBA, OPA 등



GCLP기관 네트워크 구축

- 임상시험 검체 공동분석 체계 구축
- 국내 GCLP기관과 기술교류(with, 질병청, IM 등)
- 백신 개발사와 MOU체결



생물안전3등급 시설(BL3) 운영

- '제3위험군 병원체자원' 활용 시험
- 취급병원체 : SARS-CoV-2, Monkeypox virus(Mpox)



백신 효능 평가 및 면역원성 분석법 개발 지원



분석법 기관간 검증

신규 분석법 SOP 공동개발

Vaccine Center for Assisting Safety and Technology

Good Clinical Laboratory Practice (GCLP)

Vaccine Center for Assisting Safety and Technology(K-VCAST) is a non-profit foundation under the Ministry of Food and Drug Safety(MFDS) pursuant to Article 90-2 of the Pharmaceutical Affairs Act.

Establishment purpose is to support the rapid development of domestic vaccines by providing technical support for commercialization, including the clinical sample analysis, quality control test.

It has been designated as a 'Good Clinical Laboratory Practice(GCLP)' by MFDS('23.5.8.) and is conducting immunogenicity analysis with professional personnel and facilities in accordance with the GCLP guidelines.

K-VCAST will be with you in the clinical trial sample analysis for biopharmaceutical and vaccine development.



Immunogenicity Test of Vaccines and Medicines

- Total Antibodies(ELISA etc.)
- Neutralizing Antibodies (PRNT, FRNT, PBNA etc.)
- Cytokine Analysis(ELISpot)
- Generations of Pseudovirus
- Other Analysis: SBA, OPA etc.



Build of GCLP Networking

- Establishment of a Joint Analysis System for Clinical Trial Samples
- Technology exchanges with GCLP institutions (with, KDCA, IM etc.)
- MOU signed with vaccine Developer



Operation of Biosafety Level 3(BL3) facilities

- Utilization Test of 'Risk group 3 pathogen'
- Pathogens : SARS-CoV-2, Monkeypox virus(Mpox)



Support for Immunogenicity Test Development



Inter-Laboratory Validation

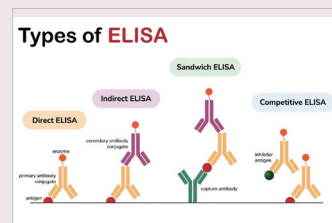
Joint Development of Standard Immunogenicity Test

결합항체가 분석

ELISA

Enzyme-Linked
Immunosorbent Assay

ELISA 분석법 종류



ELISA 분석 방법

ELISA(Enzyme-Linked ImmunoSorbent Assay)

- 항원-항체 반응을 이용하여 항원(antigen) 또는 항체(antibody)를 정량하는 분석법
- 민감도 및 특이도가 높음

• Direct ELISA

항원과 반응하는 항체에 바로 효소를 결합시켜 사용하는 분석법

• Indirect ELISA

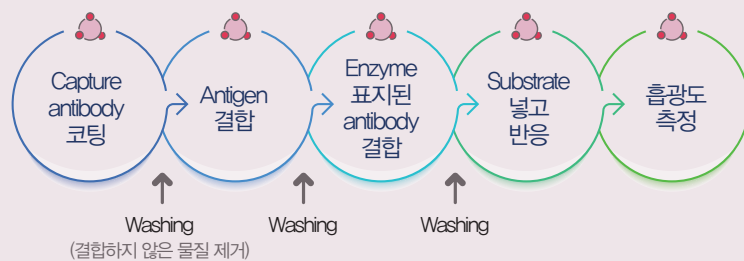
항원과 결합하는 항체(1차 항체)에는 효소가 없고, 그 항체와 결합하는 항체(2차 항체)에 효소가 결합되어 있는 분석법

• Sandwich ELISA

Capture antibody를 먼저 plate에 결합시키고, 그 항체에 항원을 결합하여 direct 또는 indirect로 반응 조사하는 분석법

• Competitive ELISA

2가지 항원(샘플 내의 비표지된 항원, 표지된 항원) 간의 경쟁적 항체 결합에 의해 이루어지는 분석법



ELISA 분석 결과(예시)



- Manufacturer : Biotek
- Model : SYNERGY H1

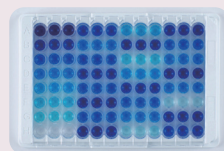


- Manufacturer : Biotek
- Model : 50TS

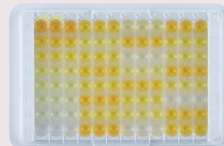
• Examples of ELISA images

	1	2	3	4	5	6	7	8
A	BLK	BLK	SPL1	SPL1	SPL9	SPL9	SPL17	SPL17
B	CTL1	CTL1	SPL2	SPL2	SPL10	SPL10	SPL18	SPL18
C	CTL2	CTL2	SPL3	SPL3	SPL11	SPL11	SPL19	SPL19
D	STD1 50	STD1 50	SPL4	SPL4	SPL12	SPL12	SPL20	SPL20
E	STD2 40	STD2 40	SPL5	SPL5	SPL13	SPL13	SPL21	SPL21
F	STD3 30	STD3 30	SPL6	SPL6	SPL14	SPL14	SPL22	SPL22
G	STD4 20	STD4 20	SPL7	SPL7	SPL15	SPL15	SPL23	SPL23
H	STD5 10	STD5 10	SPL8	SPL8	SPL16	SPL16	SPL24	SPL24

	1	2	3	4	5	6	7	8
A	0.034	0.039	0.053	0.047	0.112	0.038	0.037	0.037
B	0.034	0.041	2.712	3.102	1.542	1.698	0.077	0.084
C	0.045	0.045	0.041	0.044	0.042	0.042	0.039	0.038
D	0.04	0.039	0.056	0.041	0.039	0.04	0.038	0.041
E	0.126	0.118	2.919	3.015	0.772	0.83	0.108	0.161
F	0.038	0.039	0.045	0.044	0.045	0.042	0.038	0.039
G	0.212	0.241	2.878	2.74	1.693	1.835	0.117	0.141
H	0.039	0.041	0.047	0.047	0.04	0.041	0.038	0.039
A	0.286	0.248	3.125	3.418	1.127	1.349	0.113	0.114
B	0.039	0.04	0.043	0.052	0.042	0.043	0.038	0.038
C	0.399	0.291	2.866	3.269	1.71	1.903	0.29	0.219
D	0.043	0.04	0.045	0.045	0.044	0.044	0.039	0.04
E	0.515	0.429	3.156	3.323	2.338	2.396	0.424	0.523
F	0.041	0.047	0.05	0.047	0.052	0.046	0.04	0.041
G	0.803	1.193	3.436	3.505	2.909	2.746	1.008	1.012



Adding the stop solution

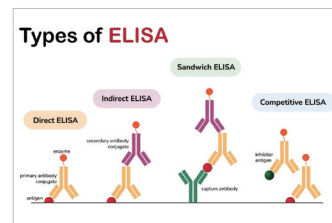


Antibody titer analysis

ELISA

Enzyme-Linked
Immunosorbent Assay

Type of ELISA



ELISA Method

ELISA(Enzyme-Linked ImmunoSorbent Assay)

- Assay for Quantifying Antigen or Antibody Using Antigen-Antibody Reactions
- Possible in a biosafety level 2(BL2) facility. High sensitivity and Specificity.

• Direct ELISA

Enzyme directly bound to an primary antibody that reacts with an antigen.

• Indirect ELISA

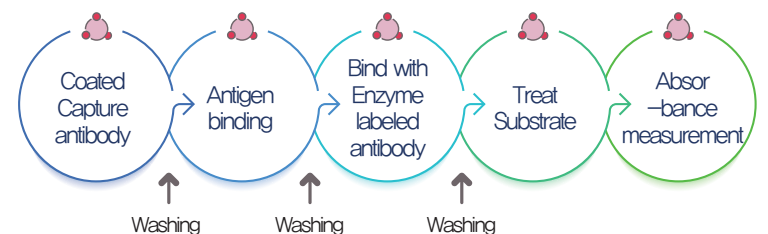
The enzyme does not bind directly to the primary antibody but attaches to the secondary antibody that binds to the primary antibody

• Sandwich ELISA

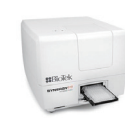
A capture antibody that binds to an antigen is bound to the plate, and the antibody is bound to the antigen to analyze direct and indirect

• Competitive ELISA

Assay performed by competitive antibody binding between unlabeled and labeled antigens in a sample



ELISA Results(Examples)



- Manufacturer : Biotek
- Model : SYNERGY H1

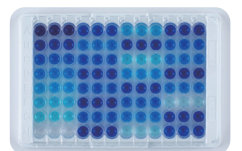


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- Model : 50TS

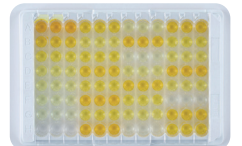
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G	0.803	1.193	3.436	3.505	2.909	2.746	1.008	1.012



Adding the stop solution



PBNA

PsV 작용 원리

```

graph TD
    A[96-Well Plate 세포 분주] --> B[검체 및 QC sample 준비]
    B --> C[Pseudovirus – 검체 반응]
    C --> D[293TT 세포 처리]
    D --> E[SEAP, Luciferase 측정]
    E --> F[중화항체가 계산]
  
```

96-Well Plate 세포 분주

검체 및 QC sample 준비

Pseudovirus – 검체 반응

293TT 세포 처리

SEAP, Luciferase 측정

중화항체가 계산

플라스미드 증폭 확인	Transfection 확인	Pseudovirus 정제
화학적발광이미지분석장치 - Manufacturer : VILBER - Model : FUSION SOLO S	형광현미경 - Manufacturer : ZEISS - Model : Primovert	초고속원심분리기 - Manufacturer : BEKMAN COULTER - Model : Optima XE-100

[illegible]

PBNA

The diagram illustrates the working mechanism of PsV (Poliovirus) and the role of antibodies. It is divided into two main sections: Non-Neutralizing Antibodies and Neutralizing Antibodies.

Non-Neutralizing Antibodies: This section shows a virus particle (labeled 'PSV') with a green outline. A red arrow labeled 'Infection' points from the virus to a cell. The cell is shown with a nucleus and a red dot, indicating infection. A magnified view of the cell shows the virus particle inside, with a red dot and a yellow starburst, indicating infection.

Neutralizing Antibodies: This section shows a virus particle (labeled 'PSV') with a green outline. A red arrow labeled 'No Infection' points from the virus to a cell. The cell is shown with a nucleus and a red dot, indicating no infection. A magnified view of the cell shows the virus particle outside, with a red dot and a yellow starburst, indicating no infection.

```
graph TD; A[Cultured Cell in 96-Well Plate] --> B[Sample and Standard preparation]; B --> C[Conjugate Pseudovirus - Sample]; C --> D[Treated with 293TT cell]; D --> E[Measure SEAP, Luciferase activity]; E --> F[Calculate Neutralizing antibody]
```

Cultured Cell in 96-Well Plate

Sample and Standard preparation

Conjugate Pseudovirus - Sample

Treated with 293TT cell

Measure SEAP, Luciferase activity

Calculate Neutralizing antibody

Plasmid amplification	Transfection	Pseudovirus separation
Chemiluminescent image analysis - Manufacturer : VILBER - Model : FUSION SOLO S	Fluorescence Microscope - Manufacturer : ZEISS - Model : Primovert	Ultracentrifuge - Manufacturer : BEKMAN COULTER - Model : Optima XE-100

[illegible]

중화항체가 분석

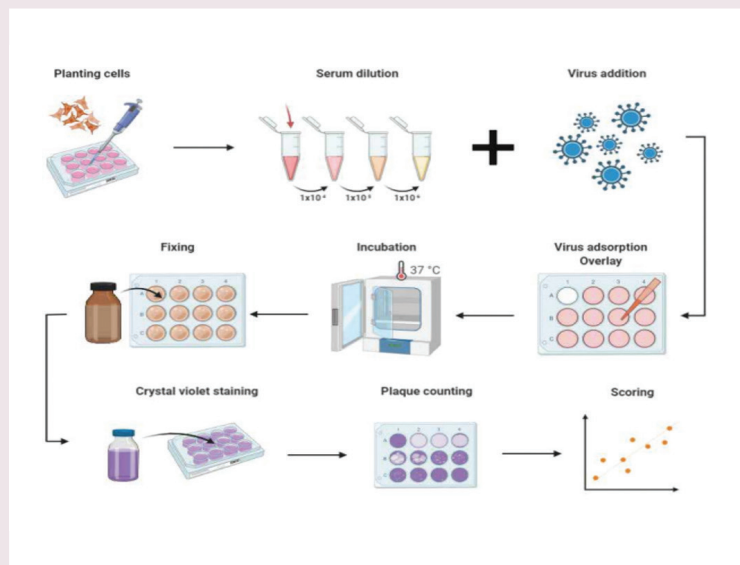
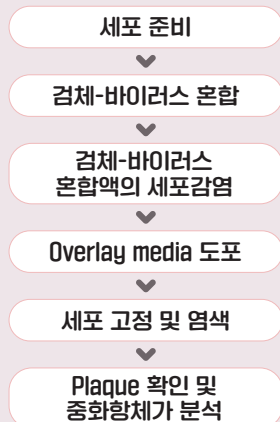
PRNT

Plaque Reduction
Neutralization Test

PRNT(Plaque Reduction Neutralization Test)

- 특이성
 - 특정 바이러스에 대한 항체의 능력을 평가하는 분석법으로 특이성이 높음
 - 백신 개발 및 면역학적 연구에서 중요하게 활용
- 정확성
 - 실제 바이러스를 이용한 실험으로 다양한 바이러스에 적용 가능
- 정량적 데이터
 - 중화하는 항체의 농도를 정량적으로 평가
 - 백신의 효과를 평가하는데 필수적

PRNT 분석 방법



PRNT 분석 결과

분석 장비

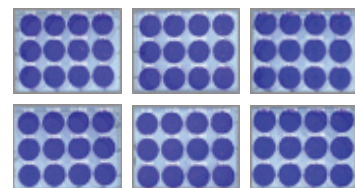


- Manufacturer : Symbiosis
- Model : ProtoCOL3

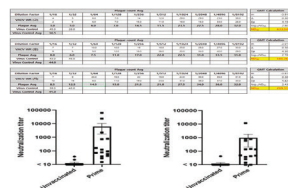
PRNT 결과

– 두창바이러스를 이용한 PRNT 결과(예시)

• 플라크 카운팅



• ND50 계산



Neutralizing antibody analysis

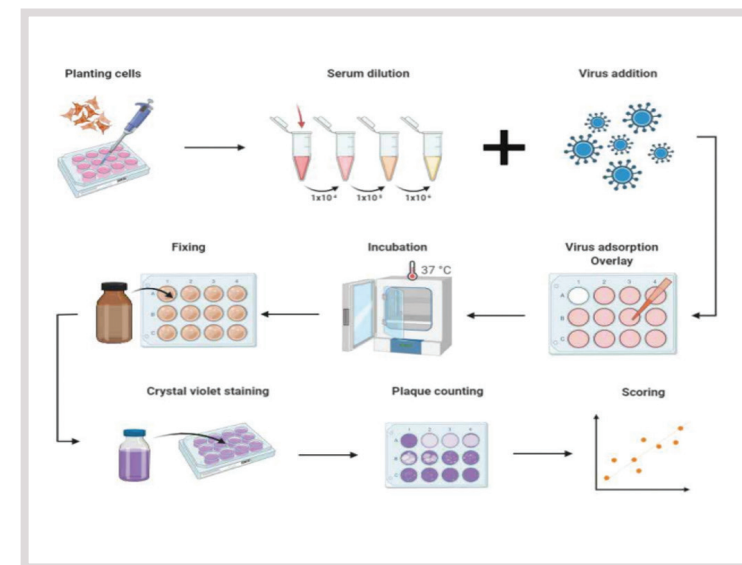
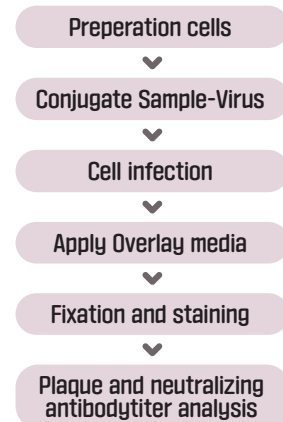
PRNT

Plaque Reduction
Neutralization Test

PRNT(Plaque Reduction Neutralization Test)

- Specificity
 - Evaluates the ability of antibodies against a specific virus
 - Used in vaccine development and immunological research
- Accuracy
 - Applicable to various viruses
- Quantitative data
 - Quantitative evaluation of the concentration of neutralizing antibodies
 - Indirectly assessing the effectiveness of vaccines

PRNT Method



PRNT Result

Equipment

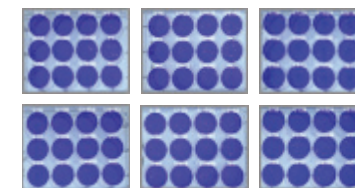


- Manufacturer : Symbiosis
- Model : ProtoCOL3

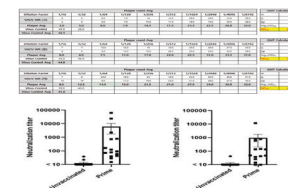
PRNT Results

– 두창바이러스를 이용한 PRNT 결과(예시)

• 플라크 카운팅



• ND50 계산

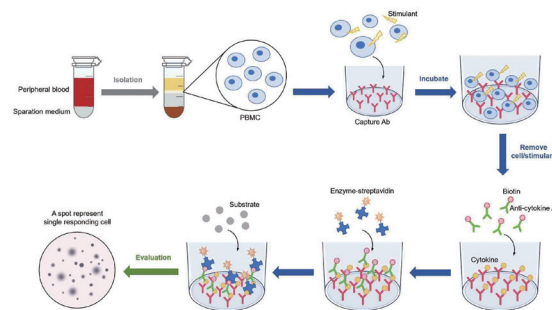


세포매개 면역반응 분석 ELISpot

Enzyme-Linked
ImmunoSpot

ELISpot(Enzyme-Linked ImmunoSpot)

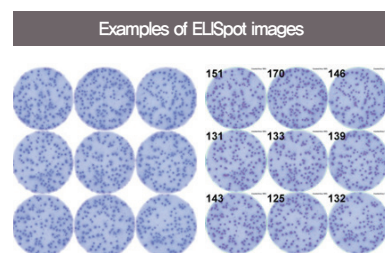
- 세포성 면역원성을 측정하기 위하여 T cell에서 분비되는 다양한 cytokine을 정량적으로 측정하여 백신의 면역원성을 평가하고 있음
- Cytokine 측정법 중 하나인 ELISpot은 antibodies를 사용하여 single cell이 분비한 cytokine을 측정하는 분석법으로 특정 세포가 분비하는 미세한 단백질 분석에 용이하며 민감도가 높음



Conventional ELISA vs ELISpot

Comparison	Conventional ELISA	ELISpot
Sample format	Supernatant	Cell culture
Measurement format	Supernatant	Single cell
T cell functional study	No	Yes
Per cell cytokine productivity	Net cytokine (Source non-distinguishable)	Cytokine per cell (Source of cytokine)
Resolution	Subtle differences non-detectable	High
Dual detection	No	Yes
Storage time	Hours	Weeks to months
Detection device	ELISA reader	ELISpot reader

ELISpot 분석 결과 및 분석 장비

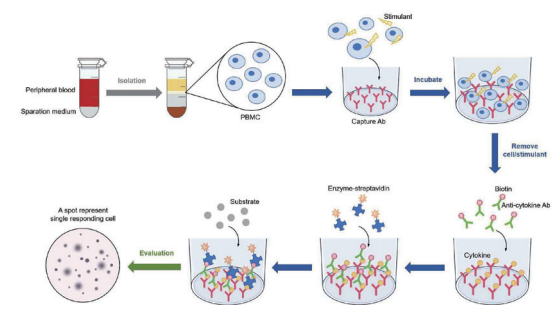


Cellular immunogenicity analysis ELISpot

Enzyme-Linked
ImmunoSpot

ELISpot(Enzyme-Linked ImmunoSpot)

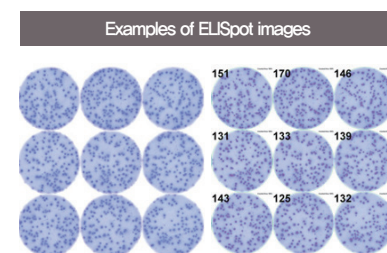
- To measure cellular immunogenicity, the immunogenicity of vaccine is evaluated by quantitatively measuring various cytokines secreted by T cells.
- ELISpot, one of the cytokine measurement methods, is an analysis that uses antibodies to measure cytokines secreted by a single cell. It's easy to analyze proteins secreted by specific cells and has high sensitivity.



Conventional ELISA vs ELISpot

Comparison	Conventional ELISA	ELISpot
Sample format	Supernatant	Cell culture
Measurement format	Supernatant	Single cell
T cell functional study	No	Yes
Per cell cytokine productivity	Net cytokine (Source non-distinguishable)	Cytokine per cell (Source of cytokine)
Resolution	Subtle differences non-detectable	High
Dual detection	No	Yes
Storage time	Hours	Weeks to months
Detection device	ELISA reader	ELISpot reader

ELISpot Results & Equipment



기능적 항체 분석

SBA

Serum Bactericidal Assay

OPA

OpsonoPhagocytic Assay

SBA, OPA

(Serum Bactericidal Assay, OpsonoPhagocytic Assay)

• SBA(Serum Bactericidal Assay)

- 혈청에서 방어면역을 측정하기 위한 분석법으로 대상균과 보체를 이용하여 항체 특이 보체면역반응을 통한 Bacteria lysis 를 확인하는 방법
- 백신접종으로 인한 양적 항체를 평가할 수 있고, 백신 접종자의 Serum 양이 많을 수록 Live bacteria 가 줄어듬

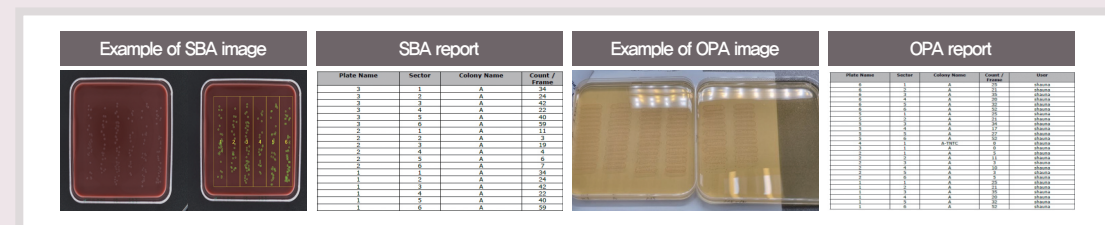


• OPA(OpsonoPhagocytic Assay)

- 혈청에서 기능적 항체가를 측정하기 위한 분석법으로 백신 접종에 의해 유도된 anti-bacteria complex의 옵소닌 식세포 능력을 측정하는 방법
- 접종자의 Serum양이 많을수록 다량의 Phagocytosis 가 발생



SBA 및 OPA 분석 결과



분석 장비



- Manufacturer : Synbiosis - Model : ProtoCOL3

Functional antibody assay

SBA

Serum Bactericidal Assay

OPA

OpsonoPhagocytic Assay

SBA, OPA

(Serum Bactericidal Assay, OpsonoPhagocytic Assay)

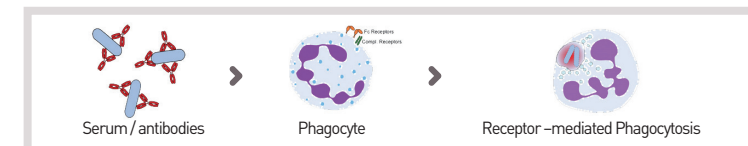
• SBA(Serum Bactericidal Assay)

- To measure protective immunity in serum and confirms bacterial lysis through antibody-specific complement immune reaction using target bacteria and complement.
- Quantitative antibodies from vaccination can be evaluated, and the higher the amount of serum in vaccinated person, the less live bacteria.

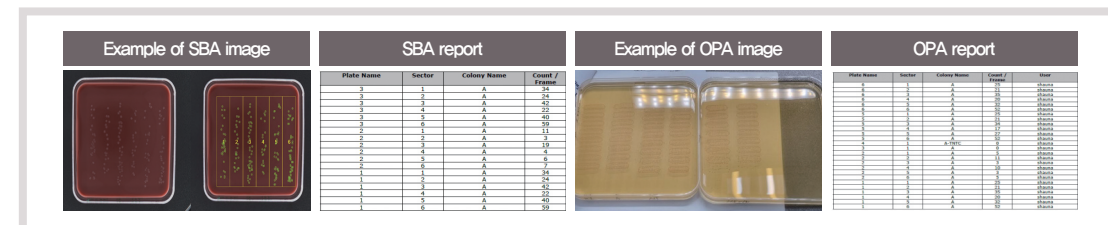


• OPA(OpsonoPhagocytic Assay)

- To measure opsonic phagocytic ability of anti-bacterium complex induced by vaccination as an assay to measure functional antibody in serum.
- The higher the amount of serum in the inoculum, the greater the amount of phagocytosis.



SBA & OPA Results



Equipment



- Manufacturer : Synbiosis - Model : ProtoCOL3

백신 등 바이오의약품
개발에서 허가까지

전주기 시험분석 역량 강화

백신 등 바이오의약품 임상시험검체 분석

백신 등 바이오의약품 개발단계 임상 시험 지원

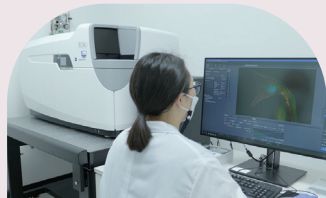
ELSpot 등 체액·세포성 면역원성 분석

2023년

- Tdap 등 4종 백신 면역원성 분석법 마련
- *Tdap, HPC, PCV, 수족구

2024년

- 2종 백신 면역원성 시험법 확립
- *HAV, 원숭이두창
- 인체조직 기증자 혈액검사
 - (NAT) 본격 시행(27개 조직은행)
 - (근거) 인체조직안전 및 관리 등에 관한 법률 시행령



전문성 Professional

다양한 분야의
전문 인력이
수준 높은 면역원성
분석 서비스 제공

Provide high-quality
immunogenicity analysis
services by experts in
various fields

자동화 Automation

분석 장비의
고도화를 통해
신속하고 신뢰도 높은
검체 분석 결과 제공

Advanced analysis
equipment provides
fast and reliable sample
analysis results

신뢰성 Reliability

임상시험검체분석
품질관리 시스템
운영을 통한
고객 만족도 향상

Improve customer
satisfaction through
operation of quality
management system

Main equipment

임상시험검체 분석장비

52종 191대(52types, 191units)

분자진단검사시스템
Molecular diagnostic system



핵산 추출 · 증폭 · 분석 등
전과정 자동화 시스템
HIV, HBV, HCV 등 검출

Digital PCR



유전자
정성적/정량적 분석

Real-time PCR



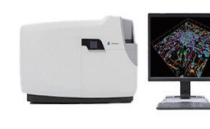
실시간 핵산 증폭 및
유전자 정성 · 정량 분석

초고속 원심분리기
Ultra centrifuge



세포, 바이러스 등의
농축, 분리, 정제

실시간 세포 이미지 분석기
Live cell imaging system



실시간 세포 관찰 및
세포기반 분석

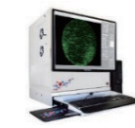
Autosampler

2025년 도입예정
(Introduction in 2025)



동시에 많은 검체 분석 시
면역원성 분석

ELISpot reader



면역형광효소분석기
(단일 세포 수준에서 cytokine
측정 및 바이러스 정량)

Microplate reader



마이크로플레이트 측정기
(Sample내 target 물질의 양을 측정)

Facilities 시설

본관동-임상시험검체분석실



- 일반실험실
- 면역화학 시험실
- 미생물 실험실
- 세포 실험실

교육동-BL3 : 생물안전 3등급(BL3) 시설 운영

- 취급 병원체(고위험병원체)
: 코로나바이러스 (SARS-CoV-2) / 원숭이두창바이러스 (Monkeypox Virus)
 - 일반구역과 물리적으로 밀폐 및 분리
 - 급기, 배기 전량 HEPA필터 여과
 - 병원체 유출방지를 위한 음압 유지
- 주요장비(터널멸균기 등 6종 8대)



터널
멸균기



이산화탄소
배양기



마이크로
플레이트 측정기



현미경



생물안전
작업대

국내 백신 등 바이오의약품의
신속한 제품화,
백신안전기술지원센터가
함께 하겠습니다.

임상시험검체분석기관

K-VCAST
재단
법인 **백신안전기술지원센터**

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102-1 Sandan-gil, Hwasun-eup, Hwasun-gun, Jeollanam-do, 58141 Republic of Korea
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