2022 **Vol.1**

Information & Technology

GC Labs

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2022 **1st** edition

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Contents

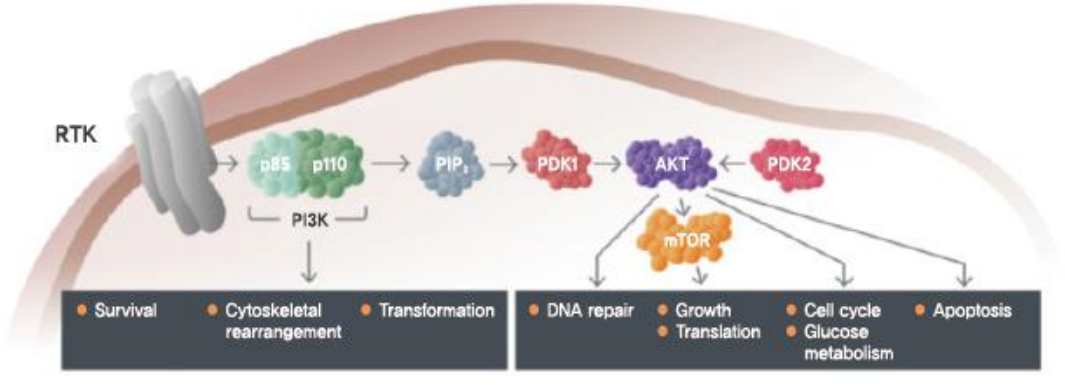
New test

Understanding the PIK3CA test

Companion diagnostic test for Alpelisib administration in patients with advanced breast cancer

Department of Laboratory Medicine
Mina Lee M.D.

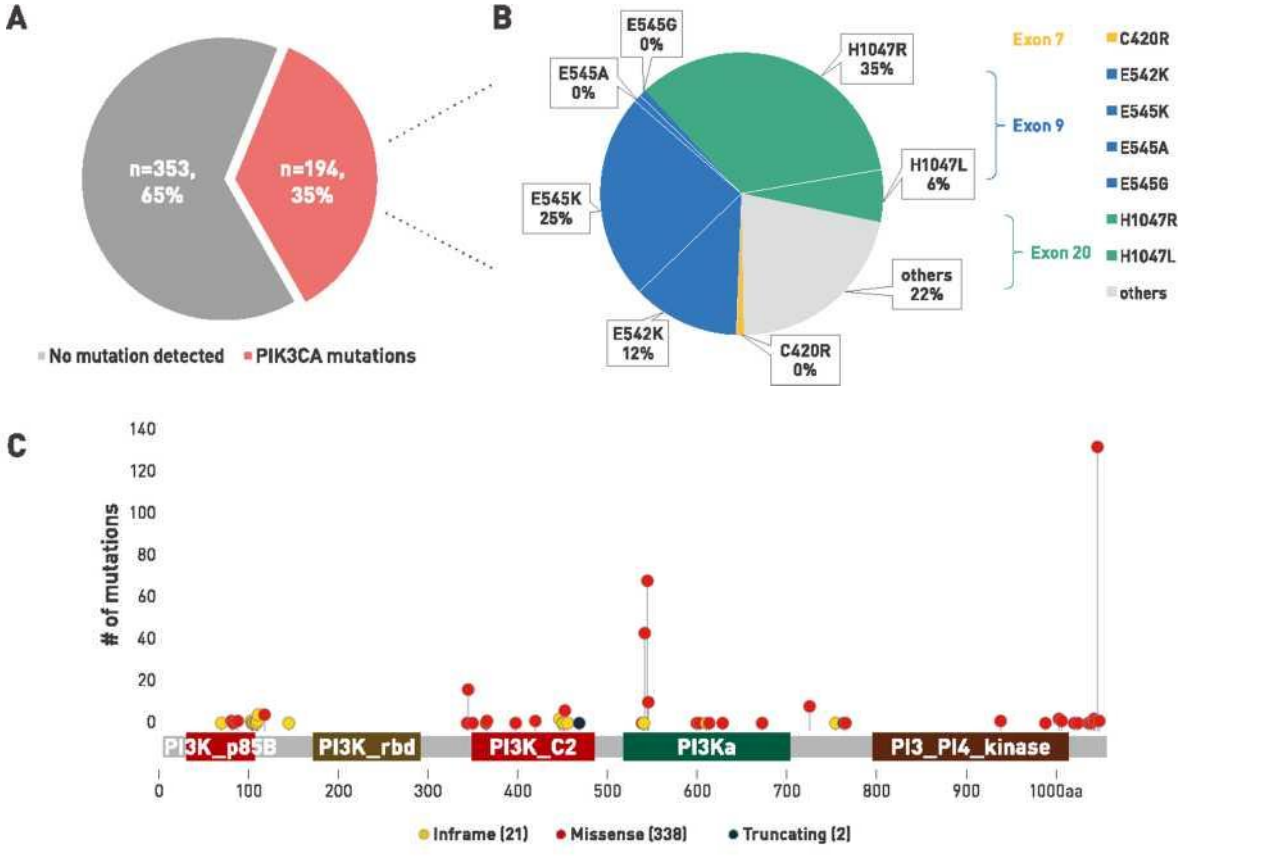
The PIK3CA gene encodes the alpha isoform (p110a) of 3-kinase (PI3K), one of the phosphorylation enzymes involved in the cell signaling pathway. Mutations in the PIK3CA gene cause the constitutive activation of the downstream path, thereby impairing the regulation of cell growth, proliferation, and apoptosis. In the case of breast cancer, they are known to be associated with disease progression and resistance to endocrine therapy.



Ref) Nat Rev Cancer. 2002 Jul;2(7):489–501.

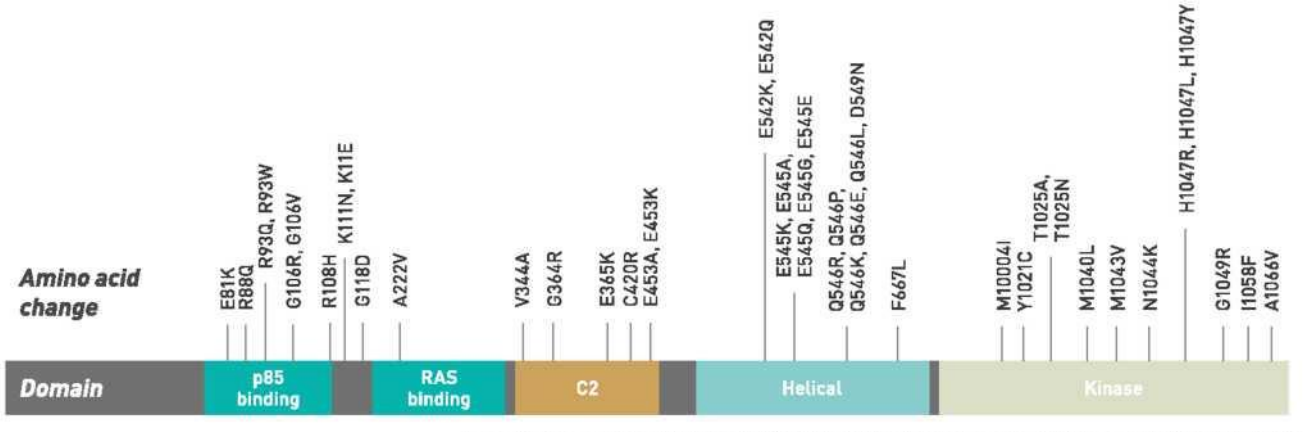
Fig. 1. PI3K pathway

The PIK3CA gene mutation is observed in various carcinomas, including colon adenocarcinoma, endometrial adenocarcinoma, lung adenocarcinoma, and breast invasive carcinoma. About 70% of breast cancer patients show hormone receptor (HR) (+) and human epidermal growth factor receptor 2 (HER2) (-), and it is known that about 40% of them have PK3CA mutation. The types/frequency and location of mutations are as follows:



Ref) Mol. Pathol. 2021, 2(1), 42–54.

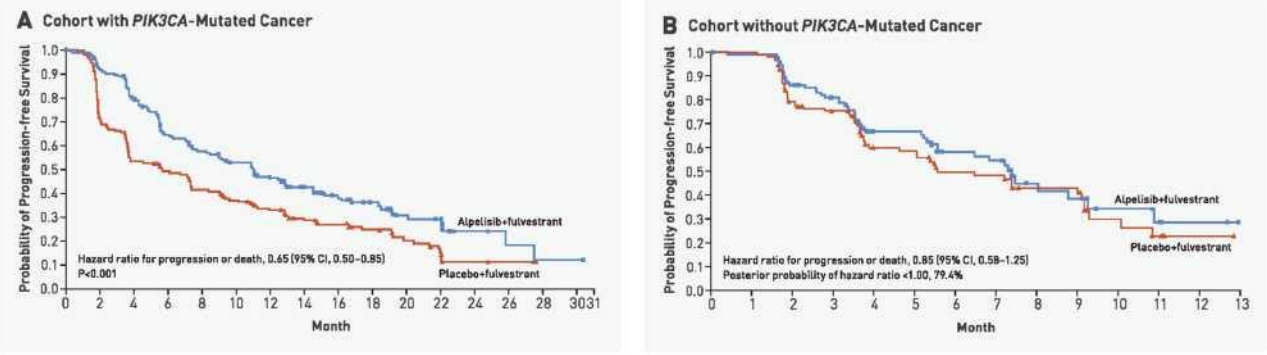
Fig. 2. Prevalence and distribution of *PIK3CA* hotspots mutations in ER (+), HER2 (-) breast cancer



Ref) Clin Cancer Res. 2011 Mar 15;17(6):1331–1340. Clin Cancer Res. 2009 Aug 15;15(16):5049–5059.

Fig. 3. Distribution of *PIK3CA* mutation

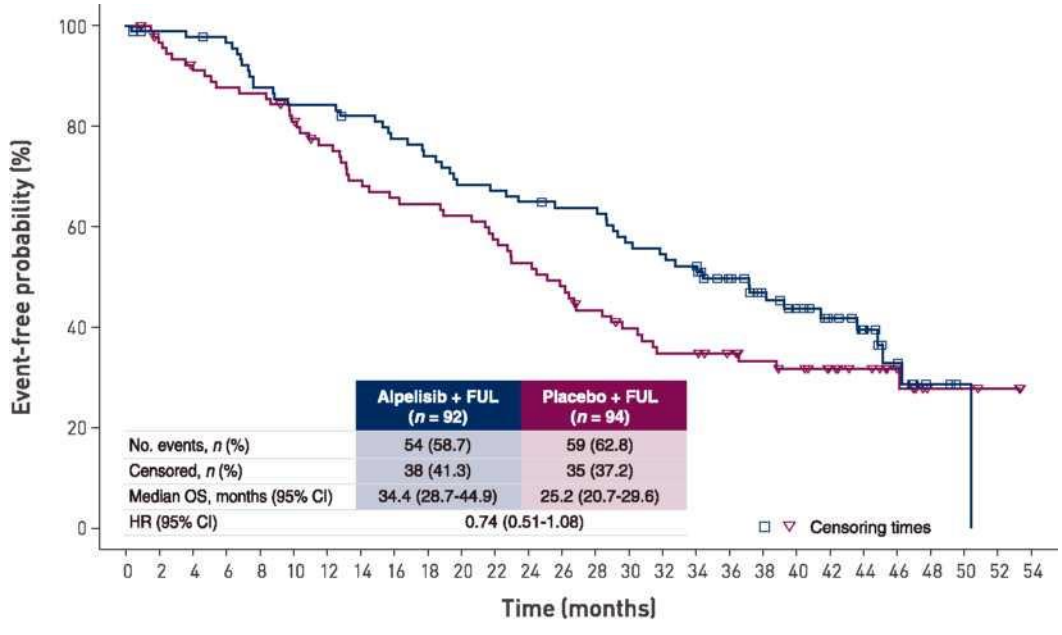
New test



Ref) N Engl J Med. 2019 May 16;380(20):1929–1940.

Fig. 4. Progression-Free Survival with Alpelisib Treatment Based on PIK3CA Mutation

A comparison of progression-free survival in the PIK3CA-mutated cancer cohort (median follow-up: 20 months) shows that the Alpelisib-fulvestrant treatment group reported 11.0 months of progression-free survival (95% confidence interval [CI], 75 to 14.5), whereas the placebo-fulvestrant group reported 5.7 months (95% CI, 3.7 to 74) (hazard ratio for progression or death, 0.65; 95% CI, 0.50 to 0.85; P< 0.001).



Ref) Ann Oncol. 2021 Feb;32(2):208–217.

Fig. 5. Overall survival in patients with *PIK3CA* mutation detected in plasma ctDNA.

In the PIK3CA mutation, higher response to and more prolonged progression-free survival were reported after Alpelisib administration, which indicates relevance to the treatment results. The consistency between samples (tissues and plasma) is within the acceptable level, which shows the validity of the technology. Now, PIK3CA test can be used to identify patients to administer Alpelisib among advanced breast cancer patients (menopause women and men) with HR (+), HER2 (-).

The positive rate in plasma can be affected by disease burden and non-tumor shedding/clearance. Therefore, the discrepancy between tissue and plasma samples is explained by tumor heterogeneity, different techniques, spatial/temporal factors, and potential plasma DNA contamination. If PIK3CA mutation is negative in plasma samples, it is also recommended to test the tissue sample for PIK3CA mutation.

Table 1. Agreement between therascreen *PIK3CA* RGQ PCR Kit plasma results and therascreen *PIK3CA* RGQ PCR Kit tissue results using the therascreen *PIK3CA* RGQ PCR Kit tissue results as reference

Measure of agreement	Percent agreement (N)	95% CI*
Positive percent agreement	55% (179/328)	(49.0, 60.1)
Negative percent agreement	97% (209/215)	(94.0, 99.0)
Overall percent agreement	72% (388/543)	(67.5, 75.2)

* 95% CI calculated using the Clopper-Pearson Exact method.

The QIAGEN Therascreen PIK3CA RGF PCR kit, approved as a companion diagnosis test kit, can identify 11 key mutations at Exon 7, 9, and 20 of the PIK3CA genes.

Table 2. Therascreen PIK3CA RGQ PCR Kit Assay Targets (List of Detectable Mutations)

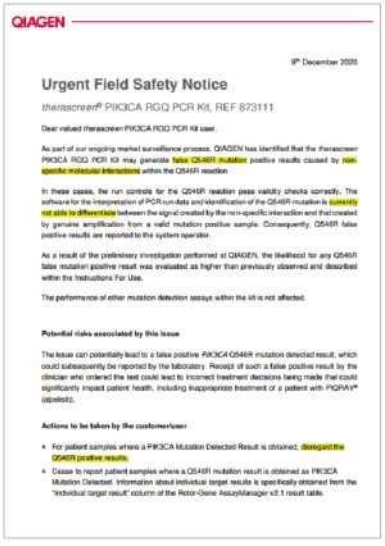
Exon	Mutation	COSMIC ID	Base change
7	C420R	757	1258 T>C
9	E542K	760	1624 G>A
	E545A	12458	1634 A>C
	E545D	765	1635 G>T
	E545G	764	1634 A>G
	E545K	763	1633 G>A
	Q546E	6147	1636 C>G
	Q546R	12459	1637 A>G
20	H1047L	776	3140 A>T
	H1047R	775	3140 A>G
	H1047Y	774	3139 C>T

New test

Cautions for Interpretation

According to the urgent field safety notice (Fig. 6) of QIAGEN, the manufacturer of the Therascreen *PIK3CA* RGQ PCR Kit, **a false positive for Q546R mutation has been confirmed.** Therefore, please note that **Q546R is not reported until Qiagen resolves this issue.** The following comment will be added to the result sheet.

*The Q546R mutation is not reported according to manufacturer's instructions, because therascreen *PIK3CA* RGQ PCR Kit may generate false Q546R mutation positive results caused by non-specific molecular interactions within the Q546R reaction (To be resolved by Qiagen).


QIAGEN

Urgent Field Safety Notice

therascreen® PIK3CA RGQ PCR Kit, REF 873111

Fig. 6. QIAGEN, Urgent field safety notice

Test Information

Test	Sample	Test day/total dates	Method	Insurance
PIK3CA (cell-free DNA) [Real-time PCR] (GC Labs code:P871)	Dedicated container *WB 8.5 mL	Tue. / 10	Real-time PCR	
PIK3CA (Tissue) [Real-time PCR] (GC Labs code:P872)	1 Paraffin block & H&E Slide sheet 5 Unstained slide sheets, 10 μm, and 1 H&E slide sheet			

▶ **Request**

PIK3CA (cell-free DNA) [Real-time PCR]

- **Molecular Genetic Test Request Form**
- Genetic Test Consent Form

PIK3CA (Tissue) [Real-time PCR]

- **Molecular Pathology Test Request Form**
- Genetic Test Consent Form

▶ ***PIK3CA* (cell-free DNA) [Real-time PCR] container*:**

Cell-free DNA collection tube

Storage/Collection: Before/after collection: room temperature / blood 8.5 ml

Handling:
- Keep at room temperature; arrive at the hospital within 7 days
- After taking blood, mix with additives around 8 to 10 times

* The above information is as of January 1, 2022 and subject to change. Please see the latest information here (http://www.gclabs.co.kr).

Information

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References

01. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer. 2002 Jul;2(7):489-501. PMID: 12094235

02. A unique spectrum of somatic *PIK3CA* (p110alpha) mutations within primary endometrial carcinomas. Clin Cancer Res. 2011 Mar 15;17(6):1331-40. PMID: 21266528

03. *PIK3CA* mutation associates with improved outcome in breast cancer. Clin Cancer Res. 2009 Aug 15;15(16):5049-59. PMID: 19671852

04. Frequency and spectrum of *PIK3CA* somatic mutations in breast cancer. Breast Cancer Res. 2020 May 13;22(1):45. PMID: 32404150

05. Alpelisib for *PIK3CA*-Mutated, Hormone Receptor–Positive Advanced Breast Cancer. N Engl J Med. 2019 May 16;380(20):1929-1940. PMID: 31091374

06. *PIK3CA* Mutation Assessment in HR+/HER2– Metastatic Breast Cancer: Overview for Oncology Clinical Practice. . Mol. Pathol. 2021, 2(1), 42-54.

07. Alpelisib plus fulvestrant for *PIK3CA*-mutated, hormone receptor-positive, human epidermal growth factor receptor-2enegative advanced breast cancer: final overall survival results from SOLAR-1. Ann Oncol. 2021 Feb;32(2):208-217. PMID: 33246021

Focus

SARS-CoV-2 Antibody Test

(SARS-CoV-2 Ab)

Department of Laboratory Medicine
Seongwook Song M.D.

Clinical Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious and pathogenic coronavirus first discovered in Wuhan, China near the end of 2019. The virus causes a severe respiratory infection called Coronavirus-19 (COVID-19). As of November 2021, 250 million have been infected, and five million have died of the disease worldwide (mortality rate: around 2%). Korea has reported 390,000 patients and 3,000 deaths (mortality rate: around 0.77%). The disease is a threat to human health.

SARS-CoV-2 Structure and Antibody Test

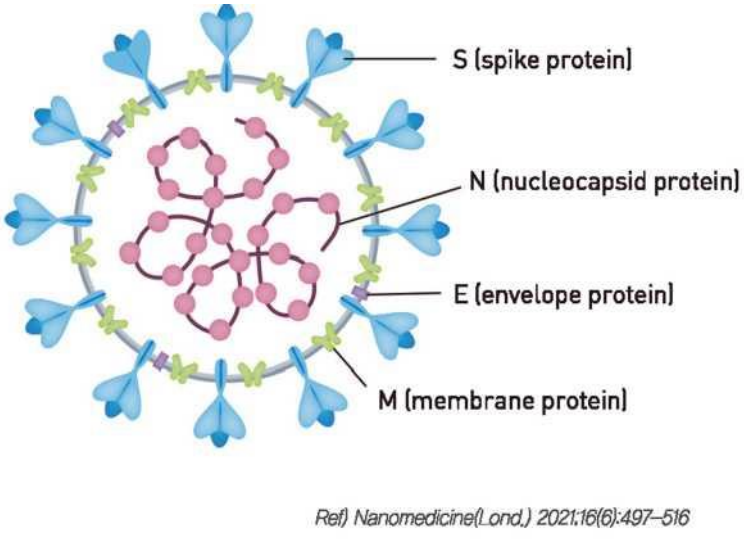

Fig. 1. SARS-CoV-2 Structure

SARS-CoV-2 is an enveloped, single-stranded RNA virus with a shell and four different proteins (S, N, E, and M) (Figure 1). The spike (S) protein allows the virus to infiltrate organs and blood vessel cells and cause infection. Most vaccine developers target this protein. S protein can be divided into two sub-types: S1 and S2. S1's receptor-binding domain (RBD) is known to directly connect with the receptor of the host cell, angiotensin-converting enzyme2 (ACE2). Nucleocapsid (N) protein is a good target for an antibody test because it takes up the highest percentage among proteins in SARS-CoV-2. The protein makes it easier to find previous infectees and study prevalence.

In the early stage of COVID-19 infection, the PCR test is the most sensitive and accurate testing method. However, the detection rate begins to decline one or two weeks after symptom manifestation, whereas the positive rate of the antibody test increases (Figure 2).

Therefore, as the world struggles with the SARS-COV-2 pandemic, we can diagnose patients accurately and begin isolation and treatment faster by using the antibody test as a supplementary to the molecular test. When performing an antibody test, it is crucial to measure the antibodies that are the most sensitive and increase early on: total antibodies (IgM and IgG). In addition, among the antibody tests developed so far, we should use SARS-CoV-2 Ab (anti-N) and SARS-CoV-2 Ab (anti-S1 RBD) in accordance with their intended purposes.

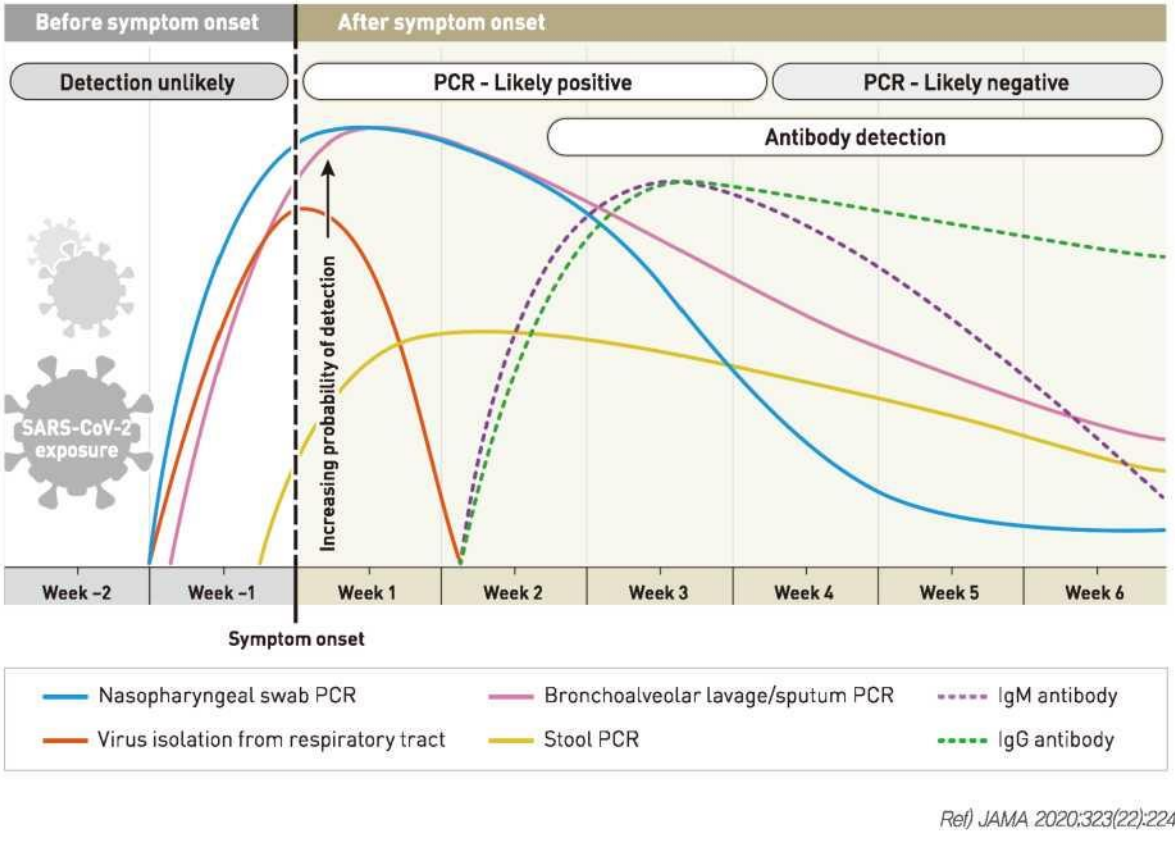

Fig. 2. Genetic Test and Antibody Detection By Time After COVID-19 Symptom Manifestation

1. **SARS-CoV-2 Ab (anti-N)**

   It is an antibody test method targeting N protein. It boasts high sensitivity and specificity. If asymptomatic and PCR-negative patients are excluded from the current infection, this positive test may indicate that the patient has been exposed to SARS-CoV-2 in the past.

2. **SARS-CoV-2 Ab (anti-S1 RBD)**

   This is an antibody test developed targeting the same S1 RBD protein as the target antigen of vaccines that have been used on the market up until recently. If a patient tests positive in this test, it may mean that the patient has developed antibodies after infection or vaccination.

Focus

Interpretation of Antibody Test Results (US CDC Guidelines)

Table 1. Test Result Interpretation Based on Vaccination Status

Vaccination status	SARS-CoV-2 Ab (anti-S1 RBD)	SARS-CoV-2 Ab (anti-N)	Interpretation*
Vaccinated	Positive	Positive	Vaccinated and previously infected
	Positive	Negative	Vaccinated and previously not infected
Not vaccinated	Positive	Positive	Not vaccinated and previously infected
	Negative	Negative	Neither vaccinated nor previously infected
Not clear on vacci nation	Positive	Positive	Not clear on vaccination, but previously infected
	Positive	Negative	Vaccinated and previously not infected
	Negative	Negative	Neither vaccinated nor previously infected

* Must refer to the cautions for antibody test result interpretation.

Cautions for Interpretation of Antibody Test Results

Due to its nature, an antibody test can be affected by various interferences (other antibodies, drugs, vitamins, etc.), resulting in a false positive or negative result. So when interpreting the result, it should be taken into consideration that antibody titers can decline over time after infection or vaccination. The physician's decision is required to use an antibody test as a supplementary to the diagnosis of patients suspected of COVID-19, and an antibody test should not be used as a sole test for diagnosis. When determining whether antibodies were generated after vaccination, a current infection should be ruled out by the absence of symptoms or, if needed, a negative PCR result. Antibody generation and immunization come from the unique reaction between the virus and the individual, and antibody generation does not always lead to immunization. As such, caution is advised when interpreting the results. In addition, the vaccination, face mask, and social distancing guidelines and rules must be observed at all times.

Test Information

Test Information	Sample (mL)	Test day/total dates	Method	
SARS-CoV-2 Ab (Anti-N) (GC Labs code:P819)	Serum 0.5	Mon-Sat/1 day	ECLIA	
SARS-CoV-2 Ab (Anti-S1 RBD) (GC Labs code:P820)	Serum 0.5		CIA	

* The above information is as of January 1, 2022 and subject to change. Please see the latest information here (http://www.gclabs.co.kr).

Information

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References

01. COVID-19 Response Guideliens 10-1,Central Disease Control Headquarters
02. Nandini S., Sundararaj S.J., Akihide R. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA 2020 ; 323(22]2249-2251
03. WHO Coro navi rus (C0VID-19) Dashboard I WHO Coronavirus (C0VID-19) Dashboard With Vaccination Data, https://covid19.who.int/
04. Hamid R., Hossein D., Hossein R., Faezeh M., Marziyeh S., Mohammad A.R. Nanotechnology against the novel coronavirus (severe acute respiratory syndrome coronavirus 2) : diagnosis, treatment, therapy and future perspectives. Nanomedicine(Lond.) 2021 ; 16(6] : 497-516
05. CDC, Interim guidelines for C0VID-19 antibody testing (updated Sep 21, 2021), https : //www.cdc.gov/coronavirus/2019-ncov/Lab/ resources/a ntibody-tests-guidelines.htmL
06. Ministry of Health and Welfare Public Notification No. 2021-266, Care Benefit Details (October 28, 2021)

Considerations for Successful Microbiome Study

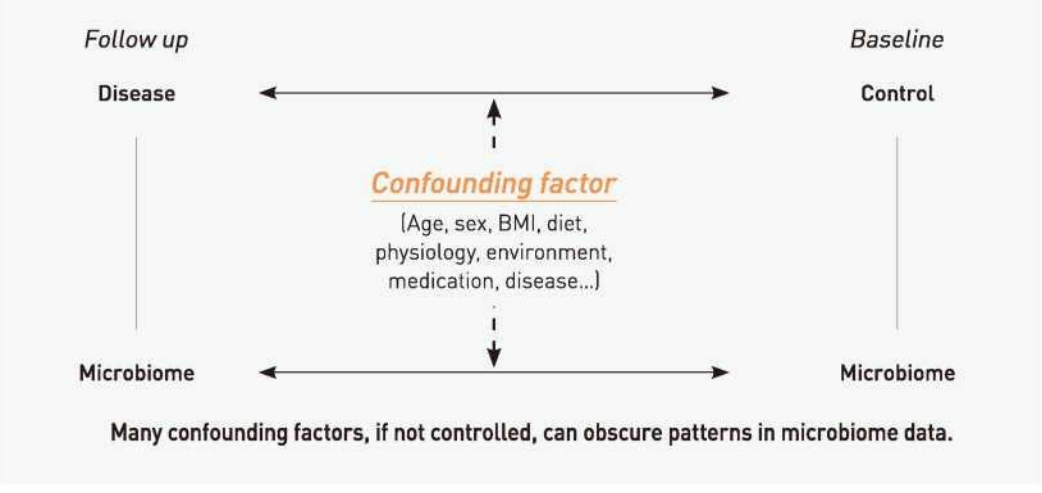
Green Gross Genome

Department of Laboratory Medicine
Juseon Song M.D.

A microbiome study consists of three phases: design, experiment, and statistics. Decisions on each phase significantly affect the final results of the study. This section reviews several considerations for a successful microbiome study, focusing on the two most important phases of the study: study design and experiment.

Study design

Microbiome studies can be classified into case-control studies and longitudinal intervention studies. Both types of studies aim to identify differences in microbiome community structure/composition in patients and follow-up test samples relative to the control group and the baseline. In this context, it is crucial to control the confounding factors when recruiting participants. Confounding factors are those affecting both dependent and independent variables. Without proper control, these factors may lead to a false correlation between dependent and independent variables.



In a microbiome study, age, sex, BMI, diet, drugs, and companion diseases affect the existence of diseases (dependent variable) and the microbiome itself (independent variable) at the same time. These interferences between the patient group and the control group should be properly controlled in order to identify the accurate microbiome marker actually related to diseases. However, it is practically impossible to control all confounding factors. In typical cases, only age, sex, BMI, drug administration, and diseases are controlled while the other factors are adjusted for using statistical methods.

Another consideration is sample size determination. In a typical study, the number of samples is determined based on the effect size between the patient group and the control group and the statistical power. However, in the field of microbiome, the body of work on effect size or statistical power is still relatively lacking. In a microbiome study, the researcher is recommended to determine the sample size based on previous studies. However, it is recommended to collect as many samples as possible because there is a wide biological variability between individuals on account of the characteristics of microbiomes.

Experiment

The experiment exerts the most significant effect on microbiome study findings than study design and statistics. Microbiome studies are typically performed using the 16S rRNA amplicon sequencing method because of its low price. The results are represented as the composition and proportion of microbiome in each sample. The findings of a 16S amplicon sequencing microbiome experiment can be affected by various processes from sampling, transportation, DNA extraction, PCR, sequencing, and BI analysis. A change to even one of these processes may significantly change the experiment results regarding the existence or proportion of microorganisms detected.

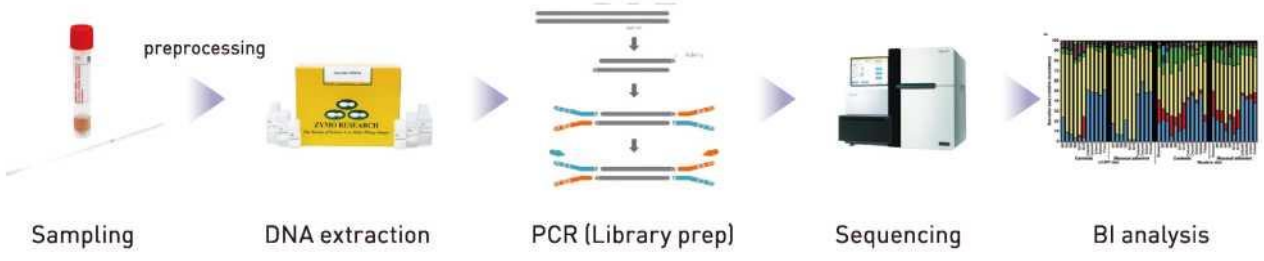


Fig. 1. 16S rRNA amplicon sequencing

So far, there have been no standard guidelines for microbiome tests. A wide variety of experimental protocols are currently applied to microbiome studies. As such, different studies have reported widely varying results. A successful microbiome study requires selecting the optimal experiment protocol. An optimal protocol means a method capable of representing the composition and proportion of microbiomes in samples with the highest accuracy.

For example, Figure 2 shows the microbiome results between urine samples refrigerated after adding boric acid and those refrigerated without boric acid. As the figure shows, the latter samples clearly show signs of enterococcus growth over time compared with the first group.

Special

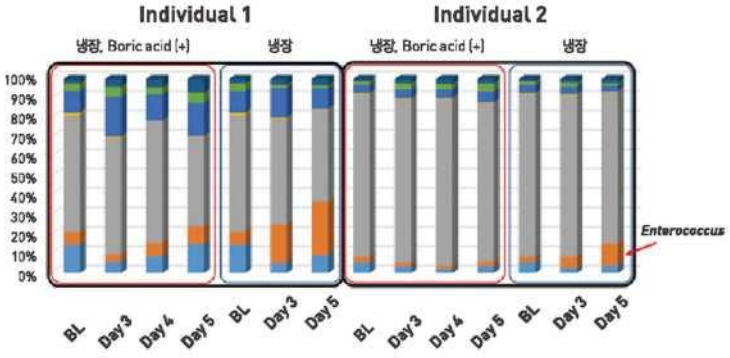


Fig. 2. Enterococcus Growth in Urine Samples Between Boric Acid and No Boric Acid Groups

The following Figure 3 compares the results according to the DNA extraction method using a mock community consisting of 6 gram-positive and gram-negative bacteria. The crux of DNA extraction from microorganisms lies in the destruction of the thick peptidoglycan wall of gram-positive bacteria. The cell wall can be destroyed either chemically with enzymes or physically with bead-beating. The result significantly varies depending on which method is chosen, the type of enzymes or bead beating, or the duration of bead beating. If DNA extraction is not done correctly, the study will yield inaccurate results even if the experiment and analysis are impeccably executed.

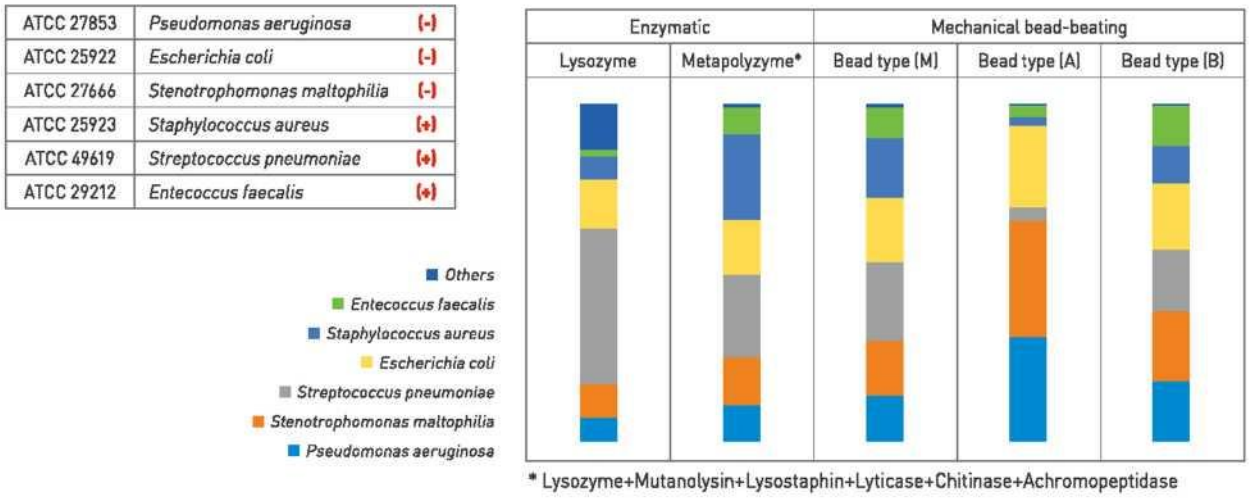
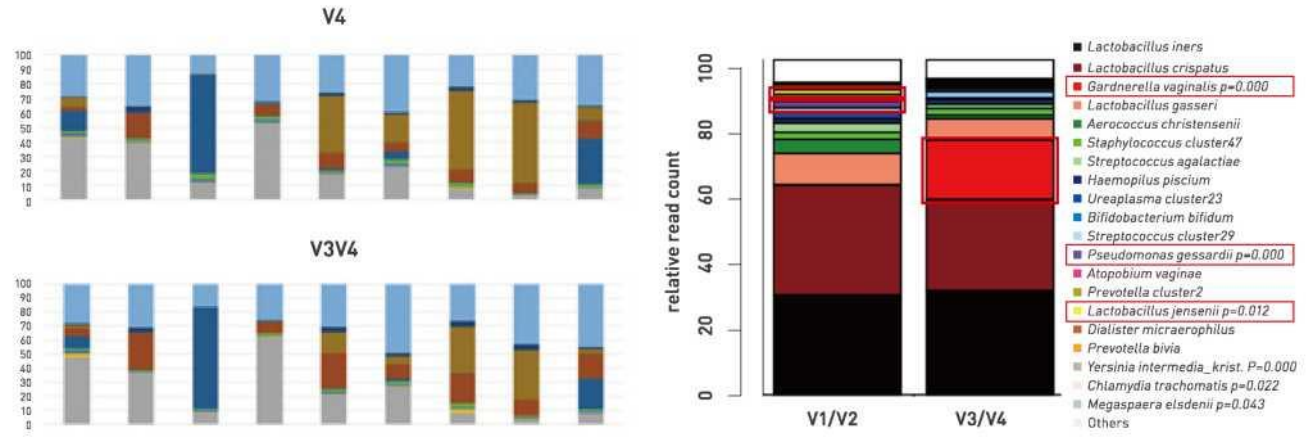


Fig. 3. Results of Different DNA Extraction Methods Using Standard Strains

The results may vary depending on which part of variable region 16s is amplified. Even if a universal primer is to be optimally combined with any bacteria, it would be impossible for the primer to combine with so many different types of bacteria and amplify them at the same level of efficiency because nucleotide sequence of the relevant part is slightly different in each bacteria. Therefore, when a PCR is performed on microbiome samples consisting of various bacteria by using different types of primers targeting different regions, the composition and proportion may vary slightly depending on the bacteria amplification efficiency of specific primers. Certain bacteria may not be detected with some primers.



Ref) Selection of validated hypervariable regions is crucial in 16S–based microbiota studies of the female genital tract, Sci Rep, 2018:8:9678

Fig. 4. Relative read count of several bacterial taxa and bacterial alpha diversity differ according to the region used.

Despite the rapid growth of microbiome studies over the last decade, there are numerous issues to be resolved before microbiomes can access the clinical realm, one of which is the standardization of experiment methods. Until the day comes, researchers should rely on their wisdom to identify and interpret each method's characteristics and pros/cons.

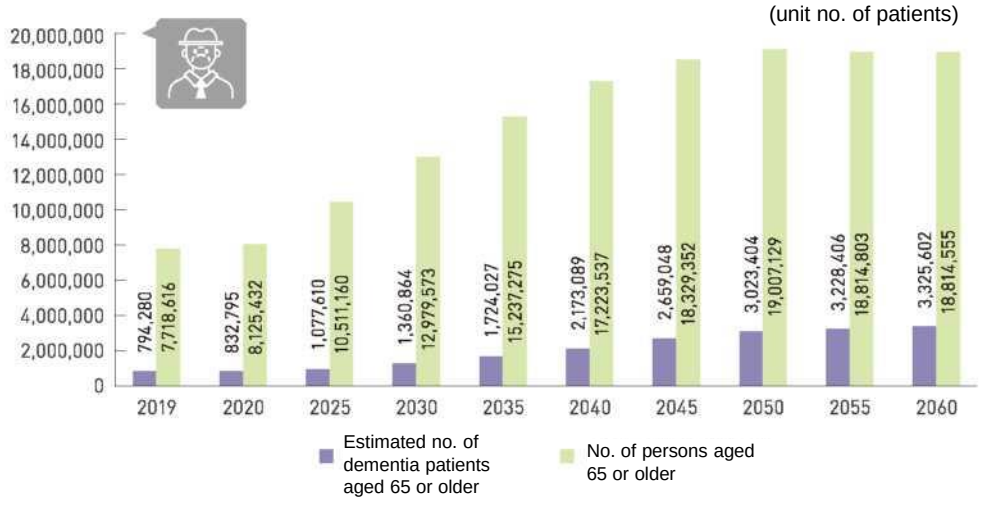
Alzheimer's Disease

1 What is Alzheimer's Disease?

Alzheimer's disease is one of the primary causes of dementia. It is a chronic brain disease where brain cell degeneration leads to gradual deterioration of cognitive functions, including memories, resulting in daily routines.

In the early stages, a patient with Alzheimer's disease experiences memory problems. As the disease progresses, they are confounded by other issues with speech functions, judgment, and other cognitive functions. Ultimately, the patient loses all ability to carry out daily functions. The progression of Alzheimer's disease is accompanied by not only cognitive deterioration but also character changes, agitation, depression, delusion, hallucination, increased aggressiveness, sleep disorder, and other behavioral and psychological symptoms. In later stages, patients may suffer from neurological disorders, including walking problems, as well as physical complications such as incontinence, infection, and bedsore.

The risk of Alzheimer's disease increases with age. The number of dementia patients has been on the rise with the country's growing population of the aged.



Ref. Central Dementia Center, Korea Dementia Status 2020

Fig. 1. Estimated Number of Dementia Patients (2019-2060)

2 What Are the Causes of Alzheimer's Disease?

The exact mechanism and cause of Alzheimer's Disease have not been identified, except that the core mechanism of the disease is excessive beta-amyloid production and its deposit on the brain, which adversely affects brain cells. In addition, a person whose immediate family suffered from the disease is more likely to suffer from Alzheimer's Disease, and genetic factors account for 40 to 50% of Alzheimer's Disease cases. APOE ε4 is one of the genes that increase the risk.

3 How do we diagnose Alzheimer's Disease?

It is crucial to gather accurate history from protectors with the most extensive knowledge about the patient. The physician verifies whether the patient suffers from changes in cognitive functions, including memory loss and when such symptoms manifested, and how. The physician also diagnoses the patients through physical examination, neurological tests, psychological tests, daily function tests, activities of daily living tests, lab tests such as blood tests, brain imaging tests, and neuropsychological tests.

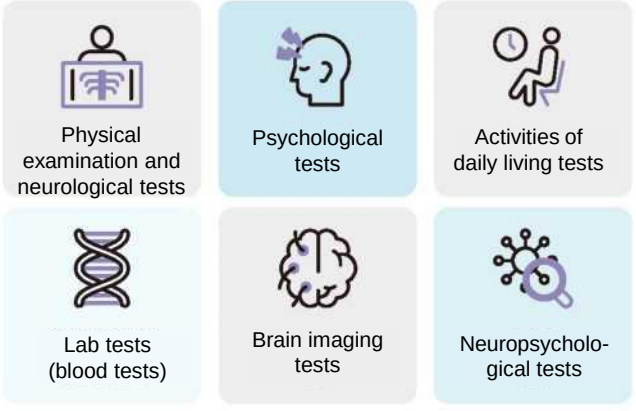


Fig. 1. Alzheimer's Disease Diagnosis Process

GC Labs offers the Apolipoprotein E (APOE) test, which is useful for predicting risks of Alzheimer's Disease, early diagnosis, and identification of various dementia. The APOE genotype test provides information on the risk factors of various diseases. In particular, as ε4 is known to be associated with atherosclerosis and Alzheimer's disease, the APOE genotype test can be used as a supplementary test useful for Alzheimer's disease diagnosis.

Test Information

Test Information	Sample (mL)	Test day/total dates	Method	
APOE genotype [Real-time PCR] (GC Labs code:S875)	EDTA WB 3,0	Mon-Fri / 3	Allele-specific PCR	

* The above information is as of January 1, 2022 and subject to change. Please see the latest information here (http://www.gclabs.co.kr).

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