# Clinical Evaluation of Genetron TERT/BRAF PCR Kit in Thyroid Cancer Patients

Study Document

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# Content

# 1 Background

Thyroid cancer is the most common endocrine neoplasm malignant, which is mainly divided into papillary thyroid cancer (PTC, accounting for 80-85%), follicular thyroid cancer (FTC, accounting for 10-15%), poorly differentiated thyroid cancer (PDTC, accounting for 5-10%), anaplastic thyroid cancer (ATC, accounting for 2-3%) and medullary thyroid cancer (MTC, accounting for 2-3%) For different pathological types of thyroid cancer, their pathogenesis, biological behavior, histological morphology, clinical manifestations, treatments and prognosis vary greatly. PTC and FTC have mild biological behavior and good prognosis, ATC and PDTC have share higher degree of malignancy and lower survival rate, and the prognosis of MTC is intermediate.

The molecular mechanism of thyroid carcinogenesis and progression is the abnormal activation of intracellular signal transduction induced by related gene changes. Among these, MAPK signaling pathway is one of the important signaling pathways for thyroid carcinogenesis and progression. The missense mutation of a single base in Exon 15 of BRAF gene (GTG>GAG) causes the replacement of valine (V) corresponding to codon 600 of translation protein by glutamate (E), which becomes activated protein kinase, and induces tumor formation by activating MAPK signaling pathway. TERT promoter mutation induces the abnormal activation of telomerase, which is a key step in thyroid carcinogenesis. It has been found that ETS (E-twenty-six) protein is a transcription factor family downstream of the BRAF-MAPK signaling pathway. When TERT gene promoter mutates, it can produce some special nucleotide sequences, which can bind to ETS protein, and cause tumorigenesis by enhancing TERT gene expression. Many studies have shown that BRAF gene V600E mutation and two mutations in TERT gene promoter region (1 295 228C>T and 1 295 250C>T) are frequently observed in thyroid cancer, and the poor prognosis of thyroid cancer patients can be effectively evaluated. Further studies have confirmed that the coexistence of BRAF and TERT promoter mutations has a synergistic effect on the invasiveness of thyroid cancer, and the two mutated tumors

are related to the high recurrence risk and increased disease-specific mortality of differentiated thyroid cancer, as well as a higher mortality trend in ATC. Therefore, TERT promoter mutation and BRAF mutation can be used as molecular markers for thyroid diagnosis.

TERT promoter mutation and BRAF mutation are closely related to clinical evaluations such as preoperative diagnosis and prognostic risk assessment of thyroid cancer, which have been clearly described in relevant guidelines. The NCCN Clinical Guidelines (Thyroid Cancer) - 2018 V3 Edition, the World Health Organization (WHO) Endocrine Tumors 2017 V4 Edition, the ATA Management Guidelines for Thyroid Nodules and Anaplastic Thyroid Cancer 2015 Edition, the Expert Consensus on Diagnosis and Treatment of Thyroid Micropapillary Carcinoma, and the Chinese Society of Clinical Oncology (CSCO) Guidelines for Diagnosis and Treatment of Persistent/Recurrent and Metastatic Thyroid Cancer in 2018 V1 Edition pointed out that, TERT promoter mutation or BRAF gene mutation showed high mutation frequency in thyroid tissue, and predicted benign and malignant tumors and adverse prognosis of patients. Among these, the ATA Management Guidelines for Thyroid Nodules and Anaplastic Thyroid Cancer 2015 Edition and the Expert Consensus on Diagnosis and Treatment of Thyroid Micropapillary Carcinoma clearly pointed out that, benign and malignant thyroid tumors cannot be determined by fine needle biopsy, and the detection of BRAF mutation and TERT promoter mutation gene can be carried out on punctured specimens, which is helpful to improve the diagnosis rate. In addition, CSCO guidelines point outed that, BRAF gene mutation and TERT gene promoter mutation are not only poor prognostic indicators for patients with thyroid cancer, but also have a greater impact on the absorption of radioactivity I in PTC patients. For PTC patients with distant metastasis, the pre-detection of BRAF V600E and other gene mutations is helpful for predicting the 131I uptake, therapeutic effect and prognosis of patients with distant metastasis. Moreover, the targeted drug dabrafenib plus trametinib can effectively alleviate ATC with BRAF mutation, while for 131I-resistant patients with BRAF mutation, dabrafenib plus131I can restore the absorption of 131I in some patients. Therefore, the detection of TERT promoter and

BRAF gene mutation is helpful for the diagnosis and clinical prognosis prediction of thyroid cancer, and convenient for making individualized diagnosis and treatment options.

The Genetron TERT/BRAF PCR Kit is used to qualitatively detect two mutations in the TERT gene promoter region (1295 2228C > T and 1295 250C > T) and BRAF gene V600E mutation (GTG > GAG) within the DNA of thyroid nodule histiocytes obtained by fine needle aspiration (FNA). Assisted fine needle biopsy is used in the diagnosis of benign and malignant thyroid nodules.

# 2 Study Purpose

The purpose of this study is to evaluate the detection performance of the Genetron TERT/BRAF PCR Kit (PCR-fluorescence probe method) by comparing the results of this kit to those of the simultaneous detection with Sanger sequencing method. Meanwhile, the clinical diagnosis performance of the Genetron TERT/BRAF PCR Kit (PCR-fluorescence probe method) will also be evaluated by comparing the results of this kit to the clinical reference standard (pathological findings or clinical diagnosis conclusions). By combining both evaluations, the intended use of the kit and the detection accuracy of the analyte can be comprehensively evaluated.

# 3 Study Design

## 3.1 Overall Study Design

### 3.1.1 Detection performance validation of test reagent

This trial will be conducted in accordance with the principles of the synchronous blind method. The selected cases will be coded, and the selected samples will be detected with the test reagent and comparison method (Sanger sequencing). When the comparison results are inconsistent with the detection results of Sanger sequencing method, the detection results of sanger will be acceptable.

### 3.1.2 Performance validation of test reagent

Among the enrolled patients, postoperative pathological results will be obtained from those who underwent surgery subsequently. Those with pathologically confirmed thyroid cancer will be considered as positive, while those without pathologically

confirmed thyroid cancer whose clinical diagnosis is non-cancer without surgery will be considered as negative. The results of the test reagents will be compared and the clinical sensitivity and specificity of the test reagents will be calculated.

**3.2 Sample Screening**

**3.2.1 Basis to Determine Sample and Sample Size**

**3.2.1.1 Sample Size of Test Performance Verification**

$$n = \frac{\left[Z_{1-\alpha/2}\sqrt{P_0(1-P_0)} + Z_{1-\beta}\sqrt{P_T(1-P_T)}\right]^2}{(P_T - P_0)^2}$$

Where, n represents the sample size; $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ are fractions of standard normal distribution, $P_0$ is the target value of the evaluation indicator, and $P_T$ is the expected value of the evaluation indicator for in vitro diagnostic reagents.

（1） BRAF gene: According to the results of preclinical studies, the target value of positive coincidence rate between test reagent and contrast reagent is up to 85% with the expected value up to 95%, the minimum sample size of positive control group is estimated to be 78; the target value of negative coincidence rate is up to 85% with the expected value up to 90%, the minimum sample size of negative control group is estimated to be 363 and the minimum overall sample size is 441.

（2） TERT gene: According to the results of preclinical studies, the target value of positive coincidence rate between test reagent and contrast reagent is up to 85% with the expected value up to 98%, the minimum sample size of positive control group is estimated to be 40; the target value of negative coincidence rate is up to 85% with the expected value up to 90%, the minimum sample size of negative control group is estimated to be 363 and the minimum overall sample size is 403.

**3.2.1.2 Sample Size of Diagnostic Performance Verification**

$$n = \frac{\left[Z_{1-\alpha/2}\sqrt{P_0(1-P_0)} + Z_{1-\beta}\sqrt{P_T(1-P_T)}\right]^2}{(P_T - P_0)^2}$$

Where, n represents the sample size; $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ are fractions of standard normal distribution, $P_0$ is the target value of the evaluation indicator, and $P_T$ is

the expected value of the evaluation indicator for in vitro diagnostic reagents.

Samples with BRAF or TERT mutation detected by the test reagent are considered as positive, and samples without mutation are considered as negative.

The results of pathological examination and clinical diagnosis will be used for comparison method. Those with pathologically confirmed thyroid cancer will be considered as positive, while those without pathologically confirmed thyroid cancer whose clinical diagnosis is non-cancer without surgery will be considered as negative.

According to relevant literature reports and preclinical study results, if the target value of sensitivity for the test reagent and comparison method is up to 50% with the expected value up to 60%, the minimum sample size of positive control group is estimated to be 194. According to previous experience, the proportion of positive diagnosis upon postoperative pathological examination is generally more than 70%. If estimated as 70%, the overall sample size of prospectively enrolled patients is 278. The target specificity value is 80% with the expected value of 90%, the minimum sample size of negative control group is estimated to be 108. If the proportion of negative results obtained from the postoperative pathological examination is estimated as 30%, the overall sample size of prospectively enrolled patients is 360. For the final overall sample size calculated based on sensitivity and specificity, the larger one (360 cases) shall prevail. Considering the estimated dropout rate of 10%, the final actual overall sample size is 400.

It is planned to carry out the trial in at least three clinical sites, with the enrollment of no less than 300 samples at each site. In case of objective reasons, when the sample size is insufficient, appropriate adjustments can be made among the sites to ensure the overall sample size. If the relevant laws and regulations change during the trial, the latest laws and regulations shall prevail, and the number of samples should be statistically significant.

**3.2.2 Inclusion, exclusion and elimination criteria**

**3.2.2.1 Inclusion criteria**

（1） Patients with clinically suspected of thyroid cancer who underwent fine needle aspiration (FNA)

（2） Samples should have appropriate basic clinical information, including: The patient's unique traceable number, age, sex, pathological diagnosis results, etc.

（3） Subjects who can provide a complete FNA sample

（4） Subjects who agreed to provide follow-up information

**3.2.2.2 Exclusion criteria**

Those who do not meet any of the above inclusion criteria will be excluded.

**3.2.2.3 Elimination criteria**

（1） The investigators consider it inappropriate to continue clinical trials, such as samples prepared without following the required procedures.

（2） Sample with incomplete sample information.

（3） Samples with DNA extraction not enough to complete the testing of test reagent or comparison method.

**3.2.3 Sample collection, storage and transportation**

**Sample collection**

Meet the hospital-based FNA puncture standard

**Sample storage and transportation**

Immediately after puncture, specimens should be placed in RNAlater protective solution. It is recommended that solid substances be isolated and tested immediately. Otherwise, it should be stored at 2 - 8 °C for no more than one month; for long-term storage, it is recommended that the isolated solid substances be stored at a temperature no higher than-70 °C for no more than one year.

**3.2.4 Sample numbering**

In principle, only one sample should be collected from one subject, and each enrolled sample of the two detection methods should have a unique clinical trial number (secondary code). After sample enrollment, before sample detection, the blinding personnel will mark the secondary code outside the DNA sample tubes according to the blinding table, prepare the Blinding Records and carefully record the comparison table between the primary code (subject enrollment number) and the secondary code as the blind codes. Blinding documents shall be kept independently by the blinding

personnel until breaking of blindness.

During experimental procedures, only the secondary code of samples is reflected.

### 3.3 Study Duration and Reasons for Determination

This clinical study will last about 12 - 36 months from drafting protocol, project establishment, ethical review, agreement signing, specimen collection, detection until the end of experiment, report completion and stamping. If samples are collected smoothly and the sample size meets the trial requirements, the trial duration can be shortened; if it doesn't go smoothly, the duration can be extended appropriately.

## 4 Statistical Method

### 4.1 General Principles of Statistical Analysis

SAS 9.4 or above software will be used for statistical analysis. The sample size is calculated with PASS16. The statistical analysis of the validity and accuracy of test results is based on full analysis set (FAS) and per protocol set (PPS), and the safety analysis is based on safety set (SS). All statistical tests will be conducted on both sides and $P \leq 0.05$ will be considered statistically significant. (Unless otherwise specified)

The quantitative variables are expressed as mean, standard deviation, median, minimum, maximum, lower quartile (Q1) and upper quartile (Q3), while the categorical variables are expressed as the number and percentage of each category.

### 4.2 Basic clinical information of enrolled samples

Descriptive statistics is collected for the basic information and data of the population corresponding to included samples. The descriptive statistics of age, sex and disease follows the general principle.

### 4.3 Positive, Negative and Total Coincidence Rates Between Test Reagent and Comparison Method

For each genetic locus, fourfold tables will be used to calculate the positive and negative coincidence rates, positive and negative predictive values, total coincidence rate and their 95% confidence intervals. Using statistical software, Kappa test is used to determine statistical significance of the two methods.

Table 1 Result Summary of Clinical Trial Comparing Test Reagent and Comparison Method

| Test Reagent | Comparison Method | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | A | B | A+B |
| Negative | C | D | C+D |
| Total | A+C | B+D | A+B+C+D |

Samples with mutant genotype detected are considered as positive, and samples without mutant genotype are considered as negative.

The calculation formula is:

Positive coincidence rate = A/(A+C)×100%

Negative compliance rate = D/(B+D) ×100%

Positive predictive value (PPV) = A/(A+B)×100%

Negative predictive value (NPV) = D/(C+D)×100%

Total coincidence rate = (A+D)/(A+B+C+D)×100%

The calculation formula of 95% confidence interval: $p\pm1.96\times[\ p(1-p)/n]^{1/2}$ (where P represents positive and negative coincidence rates, positive and negative predictive values and total coincidence rate, n presents the sample size. If p>0.9, Wilson score method will be used for correction).

**4.4 Sensitivity, Specificity and Total Coincidence Rate of Test Reagent and Clinical Reference Standard**

Table 2 Result Summary of Test Reagent and Clinical Reference Standard

| Test Reagent | Gold standard | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | A (true-positive) | B (false-positive) | A+B |
| Negative | C (false-negative) | D (true-negative) | C+D |
| Total | A+C | B+D | A+B+C+D |

Those with pathologically confirmed thyroid cancer will be considered as positive, while those without pathologically confirmed thyroid cancer whose FNA result is non-cancer without surgery will be considered as negative.

The test reagent and comparison method will be compared, but patients who fail to provide follow-up pathological results will not be included in the comparative

statistics of test reagent and clinical reference standard.

The calculation formula is:

Sensitivity = A/(A+C)×100%

Specificity = D/(B+D) ×100%

Total coincidence rate = (A+D)/(A+B+C+D)×100%

Positive predictive value (PPV) = A/(A+B)×100%

Negative predictive value (NPV) = D/(C+D)×100%

The calculation formula of 95% confidence interval: $p \pm 1.96 \times [\, p(1-p)/n]^{1/2}$ (where P represents sensitivity, specificity, positive and negative predictive values and total coincidence rate, n presents the sample size. If p>0.9, Wilson score method will be used for correction.)

**4.5 Kappa test**

Kappa test will be used to analyze the equivalence of test reagent and comparison method (clinical reference standard). Using statistical software, Kappa test will be used to compare the results of test reagent and the comparison method (clinical reference standard), and k value will be calculated. When k>0.75, the investigational reagent and comparison method show good consistency; when k>0.5, the investigational reagent and clinical reference standard show general consistency.

$$Kappa = \frac{P_a - P_c}{1 - P_c}$$ , where $P_a = \frac{A+D}{N}$ , $P_C = \frac{\frac{(A+B)(A+C)}{N} + \frac{(B+D)(C+D)}{N}}{N}$

**4.6 Clinical Evaluation Standard**

1) Coincidence rate: Compared to the comparison method, if the lower limit of two-sided 95% confidence interval for both positive and negative coincidence rates of BRAF and TERT genes are greater than the 85% of preset target value, the statistical hypothesis is valid and in good agreement with the comparison method; otherwise, the statistical hypothesis is invalid, and the agreement with comparison method is not acceptable.

2) Sensitivity and specificity: Compared to the clinical reference method, if the lower

limit of two-sided 95% confidence interval for the sensitivity of BRAF or TERT gene pooled detection is greater than 50% of the preset target value and the lower limit of two-sided 95% confidence interval for the sensitivity of BRAF or TERT gene pooled detection is greater than 80% of the preset target value, the statistical hypothesis is valid and the diagnostic accuracy is acceptable; otherwise the statistical hypothesis is invalid, and the diagnostic accuracy is not acceptable.

## 5 Quality Control Method

Quality control should be applied at every stage of the clinical trial to ensure that all data are reliable and correct. The quality control method is as follows:

(1) Qualifications of researchers involved in the study Researchers involved in the clinical trial must have professional expertise, qualifications and competences in the clinical trial, and pass the qualification examination, and staffing should be relatively fixed.

(2) Training and preliminary tests: The sponsor is responsible for the training of researchers before the start of clinical trial, so as to help clinical researchers fully understand the clinical trial protocol, the detection of investigational products, the filling of original records and forms, etc.

(3) Laboratory quality control: Clinical laboratories should be strictly in accordance with the *Measures for the Administration of Clinical Gene Amplification Testing Laboratories in Medical Institutions* (MOH Office MA [2010] No. 194 or the current effective version) and other relevant administrative regulations of molecular biology laboratories and clinical gene amplification laboratories.

(4) Only when the quality control of investigational products meets relevant requirements can the test data be valid.

(5) Clinical trial monitoring: The monitor shall make a complete monitoring plan before the start of the clinical trial, and monitor the clinical trial according to the monitoring plan to ensure that the clinical trial is carried out according to the clinical protocol, and ensure the intactness and accuracy of the original test data and records.

# 6 Provisions for Protocol Amendment

Any modification of the protocol during the trial shall be explained, and the time, reason, process and filing of the change shall be elaborated in detail, and its influence on the evaluation of the overall study results shall be demonstrated. If the investigators modify the protocol and case report form during the trial, it must be approved by the ethics committee before continuing the clinical trial.

# 7 Ethical issues and explanations involved in the clinical trial

The purpose of this clinical trial is to evaluate the performance of human TERT/BRAF Gene Mutation Pooled Detection Kit (PCR-fluorescence probe method) in clinical application. All the samples used in this clinical trial are prospective samples, which are specially collected for this clinical trial and shall be accompanied by a copy of signed informed consent form from the patient.

# 8 Data Processing and Record Storage

Investigators should record all the items on the clinical trial case report form truthfully, in detail and carefully according to the filling requirements, so as to ensure that the contents in the form are complete, true and reliable. All observations and findings obtained from the clinical trial should be verified to ensure the reliability of data and ensure that all conclusions in clinical trials come from original data. The clinical trial personnel should truly fill in relevant clinical information and the test results of clinical trials, and have them reviewed and signed by a specially-assigned person.

All original data records generated by this clinical trial shall be filed and preserved by the clinical sites, and the retention period shall be determined by the sites, but shall not be less than 5 years upon the completion of the clinical trial.

# 9 Responsibilities of Involved Parties

## 9.1 Sponsor’s responsibility

（1） Work with medical institutions to design and formulate clinical trial protocols, and sign clinical trial protocols and contracts agreed by both parties;

（2） Provide medical institutions with tested products and required contrast

reagents free of charge, and provide necessary test equipment;

（3） Provide training for study staff.

**9.2 Test facility's responsibility**

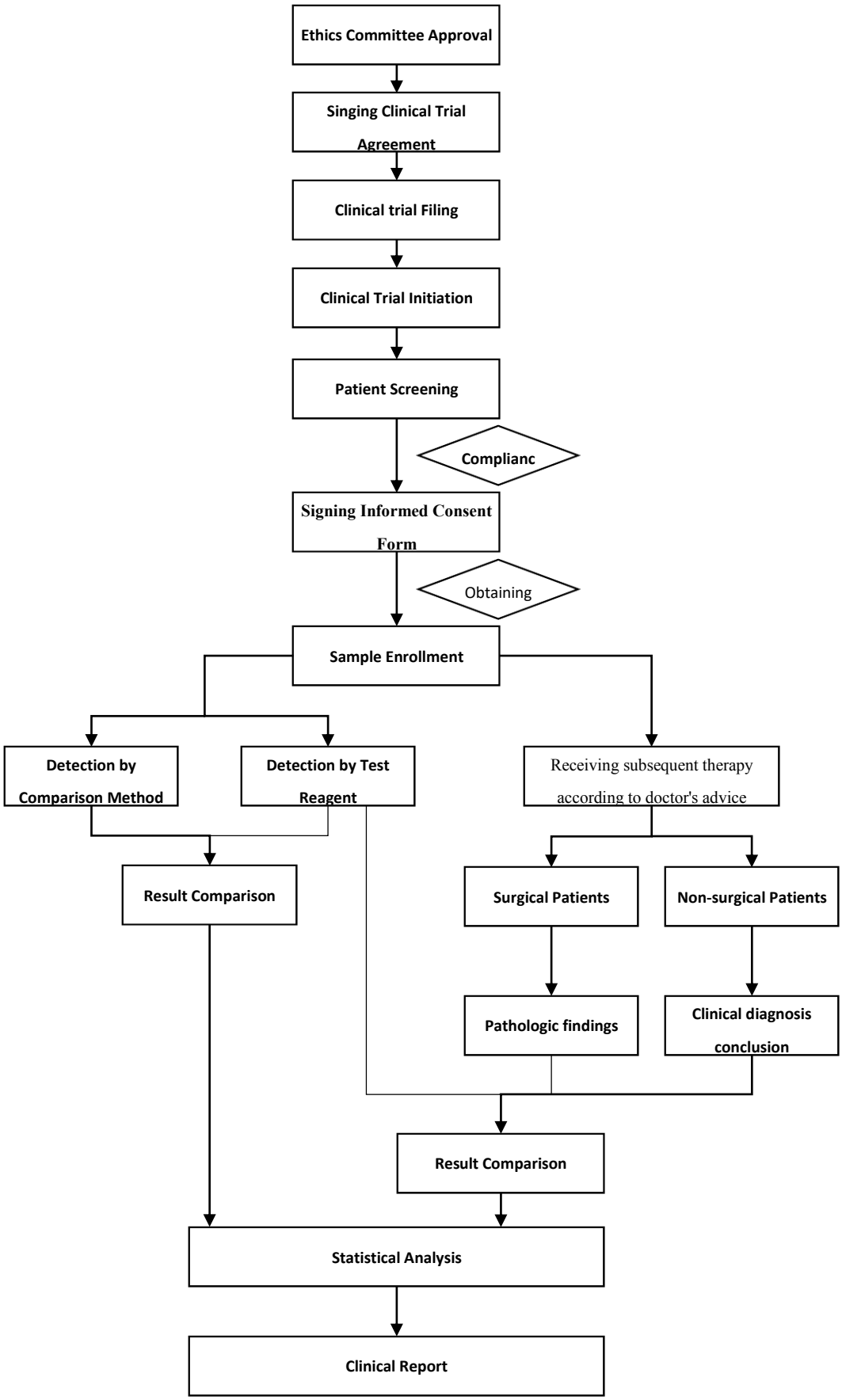
（1） Should be familiar with the relevant information provided by the implementing party and familiar with the use of the tested products;

（2） Work with the implementing party to design and formulate clinical trial protocols, and sign the clinical trial protocols between both parties;

（3） Propose clinical trial reports and be responsible for the correctness and reliability of the reports;

（4） The information provided by the implementing party shall be kept confidential, and the test results and process shall not be disclosed to any third party.

# 10 Study Technology Roadmap


Ethics Committee Approval
Singing Clinical Trial Agreement
Clinical trial Filing
Clinical Trial Initiation
Patient Screening
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Signing Informed Consent Form
Obtaining
Sample Enrollment
Detection by Comparison Method
Detection by Test Reagent
Receiving subsequent therapy according to doctor's advice
Result Comparison
Surgical Patients
Non-surgical Patients
Pathologic findings
Clinical diagnosis conclusion
Result Comparison
Statistical Analysis
Clinical Report