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Expert Consensus on Culture of Patient Derived Tumor Organoids and Organoids Based Drug Sensitivity Testing

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Abstract

The precision treatment of cancer has consistently been a significant issue in clinical management as well as medical research. With the rapid development and advances towards clinical application of organoids technology, the use of organoids derived from both normal and cancer tissues for pathophysiology studies, treatment regimen development, and drug screening has become increasingly prevalent. This document compiles insights from dozens of nationwide experts and scholars in the field of tumor organoids drug sensitivity testing. It discusses the entire process of tumor organoids drug sensitivity testing, with the intent to provide expert guidance for laboratories interested in conducting clinical testing and application research related to cancer precision therapy.

Keywords: Tumor, Personalized treatment, Patient derived tumor organoids, Drug sensitivity testing

The incidence of malignant tumors severely impacts human health and quality of life. The current treatment for cancer often involves a comprehensive approach of surgery, radiotherapy, and chemotherapy, but the therapeutic outcomes are not always satisfactory. With the advancements in precision medicine and the application of targeted therapies, there is an increased demand for

predicting the actual utility of these drugs for a patient. According to data from the United States in 2018, only 6.62% of cancer patients benefited from targeted drug therapy selected through gene sequencing [1]. Hence, there is an urgent need for clinically applicable diagnoses that serve as a basis for drug selection, thereby reducing unnecessary trial-and-error medication processes and their associated adverse effects.

Organoid technology has rapidly developed in recent years, and has attracted widespread attention in the field of precision medicine. It has been observed that tumor organoids cultured ex vivo possess a high degree of similarity to the original cancer tissue in terms of structure and three-dimensional spatial configuration. The organoids are almost identical to the tissue of origin in pathological morphology and gene expression and proteomic profiles; some even retain certain functions of the tissue or organ from which they are derived, and are widely recognized as "patient surrogates." These organoids can be utilized for drug sensitivity testing *in vitro* on behalf of patients, providing a scientific basis for the selection of anti-cancer treatments [2-3]. Consequently, the application of tumor organoid technology has provided a powerful tool for the personalization of clinical treatment strategies. As an *in vitro* model, it possesses unique advantages in predicting drug responsiveness and exploring mechanisms of drug resistance. With the aid of tumor organoid technology, it is anticipated that the goals of precision medicine will be much more achievable, tailoring therapies to individuals, and even adjusting dosages to an individual patient; thereby increasing the survival outcomes of cancer patients with potentially reduced adverse effects.

As tumor organoid culture and drug sensitivity testing technology gains momentum in the clinic, an increasing number of medical laboratories are participating in this field. After years of rapid development, the current practices have stabilized, and related kits, consumables, and equipment are readily available on the market. However, the lack of standarization in specific experimental protocols, the passage number of tumor organoids accepted for drug sensitivity testing, and the varying quality of reagents from different commercial sources have all significantly impacted the quality/interpretability of the test results. This has produced significant variation in the consistency between tumor organoid drug sensitivity testing results and clinical efficacy reported by different research teams, ranging from 71% to 95% [4-6] Therefore, standardization of clinically utilized organoid technology is urgently needed. To this end, dozens of clinical experts involved in clinical translational research utilizing tumor organoid technology in China, including experts in clinical diagnostics, medical testing, and clinical pharmacology, have assembled to exchange experimental protocols and organize laboratory

testing for experimental validation of some key parameters, thereby reaching a consensus and compiling this consensus document. In summary, the purpose of compiling this expert consensus is to standardize and normalize the process of patient-derived tumor organoid culture and drug sensitivity testing in medical laboratories, to improve the quality and validity of test reports and to ensure the comparability of test results between different laboratories and different personnel. This consensus would serve the needs of both scientific and clinical research, and accelerate the clinical translation of organoid technology to promptly improve patient outcomes.

Consensus I: Definitions of Terminology

Patient-Derived Organoids: Refers to tissue analogues created in the setting of a medical laboratory using small tissue samples from patients. These are generated and cultured *in vitro* to mimic the microenvironment of specific human tissues, resulting in three-dimensional (3D) cultures that closely resemble the original tissue in morphology, structure, genomic and proteomic profiles; and even possess limited tissue and organ functions.

Patient-Derived Tumor Organoids: Tumor organoids are tumor tissue analogues with a three-dimensional (3D) spatial configuration, derived through *in vitro* culture of tumor tissues obtained from surgery, needle aspiration, or endoscopic biopsy, or from cancer cell sediment collected from malignant pleural or abdominal effusions. Tumor organoids are highly similar to original tumor tissues in terms of morphology, structure, genomic and proteomic profiles, and they can be stably expanded in a 3D culture system.

Tumor Organoid Drug Sensitivity Testing: A technique that uses cancer tissues from surgical resection or biopsy, or t cells from malignan effusions, to establish a tumor organoids culture followed by drug sensitivity testing. Multiple anti-cancer drugs are added to the tumor organoids culture system, and the sensitivity of the drugs is measured by the degree of cell killing to screen for most effective anti-tumor drugs.

Consensus II: Ethical Requirements

Tumor organoid drug sensitivity testing currently in use remains in the clinical research and trial stage. Therefore, it is necessary to obtain both approval from relevant ethical review committees as well as written informed consent from patients before initiating the testing. The informed consent shall include, but is not limited to, acknowledgment of the risks of failure due to the limitations of current technology. Both the patient and the laboratory must be aware of these

risks/limitations. Additionally, with the patient's consent, any excess organoids obtained from culture may be used for related research after the patient's own drug sensitivity testing is completed.

The privacy of the personal information of tissue providers shall be protected. Test reports shall not be provided to any third party other than the patient themselves or their managing doctor without the patient's explicit consent. The laboratory shall make every effort to protect the privacy of the patient's medical information within the existing and future legal framework.

Consensus III: Requirements Before Specimen Collection

1.Before specimen collection, an application form shall be completed. For medical laboratory testing within a medical institution, the application form shall include the patient's name, gender, age, admission number or ID number, pathology examination number, clinical diagnosis, an overview of clinical treatment, application time (accurate to the minute), and the name and department of the attending physician with clear and legible handwriting. For testing in a third-party medical laboratory, the application form should also include the remitting hospital and contact information for the patient and the applying physician (Phone number and WeChat identification).

2.Prior to specimen collection, it is essential to review the relevant precautions for tumor organoid drug sensitivity testing specimen collection as outlined in the "Specimen Collection Manual." A dedicated specimen storage container (currently stored at 2°C to 8°C) and a dedicated specimen transfer box should be obtained. A barcode shall be printed and affixed to the wall of the specimen storage container.

3.Specimen collection shall be conducted in a treatment room or operating room, in adherence to standard aseptic techniques and protocols. To improve the success rate of sampling, analgesia and sedation may be administered as necessary, and sampling may be performed under ultrasound or CT guidance. Once the procedure is imminent, the operating physician shall verify the patient's information, confirm that the patient has followed the preparation instructions, and explain the purpose of the procedure to the patient to ensure maximum cooperation.

Consensus IV: Requirements for Specimen Collection, Packaging, Temporary Storage, and Transportation

1.Requirements for Specimen Storage Container and Barcode

(1) Two types of specimen collection containers are described as follows:

Specimen Collection Tubes: These shall have screw caps, with a diameter of not less than 2 cm and a volume of 5 - 10 ml, containing 2 - 4 ml of specimen preservation solution, suitable for preserving tumor tissues obtained from surgery, biopsy, or puncture.

Specimen Collection Bottles: These shall have screw caps, with a volume of 200-500 ml, suitable for the collection of malignant pleural or peritoneal effusions. They may be pre-filled with 10 ml-20 ml of tissue preservation solution.

(2) Barcodes shall be printed in a timely manner before specimen collection and affixed to the specimen storage containers. The slender shape of the specimen collection tubes further requires that the barcode shall be affixed parallel to the length of the specimen tube, without circling the tube wall. It shall further not obscure the view of the specimen inside, nor affect barcode scanning operations.

(3) The barcode information affixed to the specimen storage container shall be complete and clear. It shall minimally include the patient's name, type of cancer, hospital and department name, admission number or ID number, and sampling time (accurate to the minute).

2.Specimen Type and Quantity Requirement

Please refer to **Table 1** for commonly encountered specimen types and requirements for tumor organoids culture.

Table 1. Common specimen types and requirements for tumor organoid culture

Specimen Type	Collection method	Collection amount	Remarks
Fresh Tumor Tissue	Surgery	At least ≥0.5 g in total, recommended to retrieve at least 3 tissue blocks each approximately 1 cm^3 in size 。	The collection shall target areas rich in cancer cells, avoiding areas mixed with fat, mucus threads, necrotic tissue, fibrous tissue, blood clots, *etc*
	Endoscopic Biopsy	Retrieve more than 3 pieces of tissue, with a total weight of ≥0.5 g	
	Needle Biopsy	At least 3 cm in total tissue length (each piece more than 1 cm, at least 3 pieces)	
Malignant Effusion (Pleural or Abdominal Fluid, *etc*.)	Aspiration or Drainage	1) For large volumes, obtain fresh drainage fluid of 200-300 ml collected as quickly as possible; 2) For small volume, withdraw as many drainage fluid samples as possible, >50ml is recommended	1) Keep the drainage bag warm and away from light 2) Sample collection is not advisable If imaging results suggest that the amount of fluid in the patient is less than 100 ml

3.Requirements for Specimen Collection, Packaging, and Transportation of Surgically Resected

Tumor Tissue Specimens.

(1) Sampling shall be performed in a pre-disinfected operating room, strictly following aseptic techniques and protocols.

(2) The sampling physician shall plan the sampling scheme in advance, and prepare the aseptic instruments needed for sampling, such as trays, surgical scissors, scalpels, forceps, specimen collection tubes, operating room marker, *etc*.

(3) Surgically remove tumor tissue.

(4) Tumor tissue specimens shall be collected within 30 minutes to avoid changes in tissue microenvironment and ischemia that lowers tissue viability. When sampling, the surgically removed tumor tissue shall be cut open with the largest possible cross-section. If the tumor tissue is relatively small, the central area shall be obtained for testing; if the tumor tissue is relatively large, the edge area, adipose tissue area (bright yellow), muscle area (red), necrotic area (yellow or black), and highly tough and reflective fibrous area (white or gray) should be avoided. It is recommended to select the slightly hard, non-tough, and non-reflective area rich in cancer cells (light pink or pink) for sampling.

(5) The sampling amount shall be ≥0.5 g. It is recommended to take at least 3 pieces of tissue blocks of about 1 cm^3 in size each. Sufficient tissue collection is necessary for the subsequent establishment of organoids culture.

(6) After specimen collection, it is necessary to wash off any blood and/or mucus attached. Prepare 2-3 small cups containing more than 10 ml of sterile saline, immerse the resected tissue samples in cup 1 first, gently shake the cup for 10 seconds, and let the tissue blocks rotate and float; retrieve the tissue samples and place them into cup 2, and repeat the washing process. If the samples are still not sufficiently clean, repeat the washing process in the third cup. Finally, Retrieve the tissue blocks and place them on sterile gauze to absorb excess liquid.

Note: The washing process should not be of overly long duration; the tissue blocks must be transferred to the specimen preservation solution as soon as possible to maintain viability.

(7) Deposit the washed tissue samples into a specimen collection tube and gently push them to the bottom of the tube to ensure complete immersion in the specimen preservation solution inside the tube. Close the tube cap, tighten it, check to ensure no leakage occurs, and then seal the tube cap with sealing film.

Note：2 tubes are needed for 3 pieces of 1 cm^3 tissue samples.

(8) Reconfirm that the information on the specimen collection tube containing the tissue samples is

consistent with the "Application Form". Place the specimen collection tube and "Application Form" together into the specimen transport bag (a transparent plastic bag), seal the bag tightly, and then place it into the special specimen transfer box.

Note: The specimen collection tube should not be in direct contact with the ice box inside the transfer box. It is best to wrap the specimen transport bag with a thick cotton cloth and place it in the transfer box with a plastic foam between the bag and the ice box. The temperature inside the specimen transfer box should be controlled at 2°C to 8°C, and a temperature logger shall be placed inside. Then, close and seal it, and transport it to the laboratory immediately.

4.Requirements for Specimen Collection, Packaging, and Transportation of Puncture and Biopsy Specimens

(1) Biopsies shall be carried out in a pre-disinfected treatment room or operating room, strictly following standard aseptic techniques and protocols for sampling.

(2) Puncture or biopsy sampling may be performed under ultrasound or CT guidance (where necessary). Perform puncture at a site confirmed to be the location of the tumor. 2 to 3 tissue samples shall be obtained, ensuring the total length of the tissue specimen is ≥3 cm. If endoscopic biopsy is performed, more than 3 pieces of tissue shall be obtained, with a total weight of ≥0.5 g.

(3) Deposit the biopsied samples into a specimen collection tube and gently push them to the bottom of the tube to ensure complete immersion in the specimen preservation solution inside the tube. Close the tube cap, tighten it, check to ensure no leakage occurs, and then seal the tube cap with sealing film.

(4) Double check sample information, same as per 3(8).

5.Requirements for Specimen Collection, Packaging, and Transportation of Malignant Effusion Specimens

(1) Sampling shall be carried out in a pre-disinfected treatment room or operating room, strictly following standard aseptic techniques and protocols.

(2) Aspiration sampling: The patient adopts an appropriate position to fully expose the puncture site, and after surface disinfection, a large-volume syringe with a puncture needle is inserted into the effusion cavity and the effusion is slowly aspirated, and gently transferred directly into the specimen collection bottle. The bottle cap is tightened and sealed with sealing film.

(3) Drainage sampling: After the surgery, insert the drainage tube, disinfect the interface, and connect it to the drainage bag.

If the effusion volume is large and the drainage is fast, once 200ml-300 ml of drainage fluid is

collected, close the drainage tube with clips, disinfect the drainage port of the drainage bag, and transfer the drainage fluid from the drainage bag into the specimen collection bottle. Disinfect the opening of the specimen collection bottle, cover, tighten it, make sure there is no leakage, and seal the opening of the bottle with sealing film.

Note: If the amount of effusion is small, drainage is not recommended, but aspiration can be used for sampling. The volume of the specimen collected shall nevertheless be more than 50 ml. If imaging examinations indicate that the patient's effusion volume is less than 100 ml, sampling is not recommended.

(4) Double check sample information, same as per 3(8) .

6.Requirements for the Time Limit of Specimen Temporary Storage and Transportation

Tumor organoid drug sensitivity testing requires the use of viable tissue cell specimens, which may quickly lose viability after being removed from the body. Although the specimen collection tubes and bottles come pre-filled with preservation solution that minimizes factors detrimental to cell survival, it is crucial to strictly control the time from when the specimen is removed from the body until it reaches the laboratory for initial processing. In short, this duration shall be minimized.

Generally, after specimen collection, it shall be immediately transported to the laboratory for testing in an environment with a temperature range of 2°C to 8°C. However, there may be situations where the sample cannot be delivered immediately. In such cases, the specimen collection tubes or bottles should be temporarily stored in the middle of the refrigerated compartment of the operating room refrigerator (with a temperature of 2°C to 8°C), avoiding contact with the refrigerator walls to prevent accidental freezing of the specimen tubes/bottles. Under these circumstances, it is not recommended to store the specimens for more than 6 hours under these circumstances.

In special circumstances where specimens require long-distance transportation to reach the laboratory, it is advisable to use a transfer box and increase the number of ice packs inside the box. Multiple layers of thick cotton cloth should be used to insulate the specimen transfer bag from the ice packs. The duration for long-distance transportation shall not exceed 2 to 5 days (based on the duration claimed in the instructions for the specific brand of specimen preservation solution). In summary, the longer the time the specimen is out of the body, the lower the viability of the cells; it is therefore crucial to expedite transport to the laboratory.

Consensus V: Requirements for Specimen Recept

After the specimen arrives at the laboratory, it is necessary to immediately handle the receipt procedures to preliminarily determine whether the specimen is qualified.

1.Temperature check: immediately open the specimen transfer box, determine the temperature inside the box through use of the temperature logger, and record it on the "Specimen Receipt Form."

2.Sample identity check: remove the cotton bag, extract the specimen collection tube or bottle, and validate the barcode information on the specimen collection tube/bottle for consistency with the information on the "Application Form" inside the box.

3.Sample condition check: examine and record the condition of the specimen collection tube/bottle, whether it is effectively separated from the ice box, whether freezing has occurred, the color of the specimen, whether the specimen preservation solution is turbid, and whether there are any suspended solids or flocculent substances in the liquid.

4. Specimen suitability check: The quality of the specimen is crucial to ensure the success of the experiment. When the specimen arrives at the laboratory for handover, it is necessary to make a preliminary judgment on whether the specimen is qualified or may be classified as a compromised specimen. Compromised specimens must be clearly marked and the reasons for the compromise recorded on the "Specimen Receipt Form."

The definition of a qualified specimen is as follows: ① The amount of specimen collected meets the requirements of Table 1; ② The collection site is from a cell enriched areas of the tumor tissue, not adipose tissue, necrotic tissue, fibrous tissue, or blood clots; ③ The temporary storage time in the clinic and the transportation time are less than the effective preservation time declared by the specimen preservation solution; ④ The specimen has always been maintained at a temperature of 2℃～8℃ during temporary storage and transportation, and has not been in direct contact with ice or frozen; ⑤ The specimen is fully immersed in the specimen preservation solution; ⑥ The patient has not received chemotherapy treatment within 3 months.

Specimens that fall under any of the following circumstances are considered compromised: ① Insufficient specimen quantity; ② Failure to collect from cell enriched areas, or the presence of a large amount of adipose tissue, necrotic tissue, fibrous tissue, and blood clots; ③ The total duration of storage in the clinic and transportation exceeds the effective preservation time

declared by the specimen preservation solution; ④ The temperature of the specimen during temporary storage and transportation exceeded requirements for a considerable period (more than 30 minutes), or the specimen was in direct contact with ice or frozen; ⑤ Separation of the specimen from the preservation solution; ⑥ The patient had received chemotherapy within 3 months; ⑦ Failure to adhere to aseptic techniques during collection, resulting in contamination of the specimen by environmental bacteria, viruses, or fungi other than the collection site.

Note: tumor organoid drug sensitivity testing is important for enhanced clinical management of patients, and specimens may not be re-obtainable. With the informed consent of the patient and the physician, compromised specimens may be allowed to proceed through the testing process and undergo subsequent standardized operations. Maximum effort shall be taken to salvage such specimens, successfully culture organoids, and complete the drug sensitivity testing experiment.

5.Immediately send the specimen transport bag and "Specimen Receipt Form" through the transfer window into the pre-treatment laboratory.

Consensus VI: Requirements for Specimen Pre-treatment

After receiving the specimens, the pre-treatment laboratory shall process the specimens as expeditiously as possible, and aseptic techniques shall be maintained throughout the process.

1. Specimen transfer and preliminary treatment: Depending on the size of the specimen, transfer it to a culture dish with a diameter of 3.5 cm or 10 cm, which has been pre-filled with 1 ml to 2 ml of DPBS-PS at a temperature of 2℃ to 8 ℃.

2. Volume measurement: Place the culture dish against a ruler to measure the length, width, and height of the tissue specimen to calculate its volume. For puncture specimens and certain special specimens, only the length and width are usually measured, with the height considered equal to the width for estimating the volume of the specimen. Estimated volume (cm^3) = 1/2 × a × b^2, where a is the length (cm), and b is the width (cm) [7].

3. Information recording: Record the specimen by photographing and completing the relevant information registration on the "Specimen Receipt Form" to ensure the accuracy and completeness of the specimen information.

4. Requirements for Pre-treatment of Tissue Specimens

(1) Carefully check the appearance and texture of the tissue specimen, remove adipose tissue (yellow), fibrous tissue (white hard lumps), necrotic tissue (brown or black), and muscle tissue (dark red), and remove mucus threads, only retaining the pink or grayish-pink tissue that is slightly hard and has no obvious luster, which is rich in tumor cells. If it is impossible to discern tumor tissue from other tissues, this step may be omitted.

(2) Based on the size of the specimen, place it into a 50 ml Falcon tube containing 15 ml of pre-chilled DPBS-PS at 2°C to 8°C for washing. Typically, this washing procedure shall be repeated 3 to 5 times until the washing solution is clear.

(3) Gently aspirate the DPBS-PS, transfer the specimen to an EP tube or a new culture dish, and add Digestion Solution I (refer to the reagent kit manual for amount needed). Use scissors to cut the tissue specimen into 0.5 to 1 mm^3 in size. The size shall be uniform to ensure consistency in processing.

(4) Use a Pasteur pipette to transfer the chopped tissue to a 15 ml Falcon tube, add Digestion Solution I (refer to the reagent kit manual for amount needed), digest at 37°C on a shaker, and observe under the microscope every 15 minutes to check if there are large clusters of cells released.

(5) After the digestion with Digestion Solution I is finished, add 8 ml of 4°C pre-chilled DPBS-PS to terminate the digestion, centrifuge at 400×g for 5 minutes, discard the supernatant, and then add Digestion Solution II (refer to the reagent kit manual for amount needed), digest at 37°C on a shaker (refer to the reagent kit manual for digestion time).

Note： If a large number of small cell clusters (approximately 30 μm in diameter) have been observed after the previous step, and the number of large cell clusters (diameter greater than 50 μm) is relatively low, this step may be omitted. However, if there are many large cell clusters, continue with this step to further digest the cells.

(6) After the digestion is finished, add 4 ml of 4°C pre-chilled DPBS-PS to terminate the digestion, centrifuge at 400×g for 5 minutes, discard the supernatant, and collect the cell pellet.

(7) Add 2 ml of 2°C~8°C pre-chilled DPBS-PS to resuspend the cells, filter the cell suspension with a 100 μM cell sieve, and transfer the collected filtrate into a new 50 ml Falcon tube. Centrifuge at 400×g for 5 minutes.

(8) Discard the supernatant. If there are excess red blood cells in the cell precipitate suspension (the cell precipitate appears red), add red blood cell lysis solution (refer to the reagent kit manual for amount needed), incubate at room temperature for red blood cell lysis (refer to the reagent kit

manual for incubation time).

(9) Add 4 - 6 ml of DPBS-PS, resuspend the cells, centrifuge at 400×g for 5 minutes, discard the supernatant, and resuspend in 1 ml of 2°C~8°C pre-chilled DPBS-PS.

(10) Cell counting: Take a small amount of cell suspension and use an automatic cell counter to count the number and viability of cells and cell clusters per ml of cell suspension. Alternatively, manually fill the counting chamber slide, calculate the concentration of cells and cell clusters under the microscope (the counting chamber has a large square with a side length of 1μm and a height of 0.1μm, a volume of 0.1μm³), and then use the Trypan Blue staining method to examine the cell viability under the microscope.

(11) Calculate the total number of harvested viable cells and cell clusters, and then adjust cell concentration to 4000 to 6000 cells/μl with the complete culture medium supplemented with matrix gel at 1:2 ratio.

Note 1: This step needs to be kept at 2°C-8°C throughout the process.

Note 2: If the total number of harvested cells and cell clusters is sufficient, you can directly inoculate the drug sensitivity plate for organoid establishment and prepare the drug sensitivity experiment with the primary organoids; if the total number of harvested cells and cell clusters is not enough, inoculate the amplification plate for organoid establishment, and after harvesting sufficient organoids, inoculate on the drug sensitivity plate.

Note 3: Considering the possibility of mutations in the *in vitro* culture and passage of tumor organoids, the more passages performed, the higher the risk of mutation. Therefore, tumor organoids subjected to drug sensitivity experiments should not be passaged excessively. However, tissue obtained from puncture or biopsy sources is usually limited, and the number of cells and cell clusters collected after specimen pre-treatment is not sufficient to meet the demand for the multiple (tens of) drug sensitivity tests expected in the clinic. Therefore, the laboratory needs to carry out passage amplification before the drug sensitivity experiment. In addition, some tissues obtained from surgical resection, due to poor sampling sites, mainly consist of fibrous tissue, adipose tissue, muscle tissue, and even necrotic tissue, and the number of harvested cells and cell clusters may also be insufficient, which requires passage amplification of organoids. To ensure the maximum consistency between the cultured organoids and the original organs, and considering the limitations of current technical conditions, it is recommended to prioritize the use of primary organoids (P0) or first-generation organoids (P1) for drug sensitivity experiments. Second-generation organoids (P2) can be used with discretion, and it is not

recommended to use third-generation organoids (P3) or later generations.

5.Requirements for pre-treatment for malignant serous cavity effusion (pleural and abdominal fluid)

(1) Physical examination: Remove the pleural and abdominal fluid specimen collection bottle from the specimen transport box, observe the color, judge the transparency. Gently rotate and check for coagulation, clots, and flocculi, and record observation from the physical examination.

(2) Smear examination: Mix the effusion fluid and aliquot a small amount of the specimen to produce a smear for immediate microscopic examination to observe for presence of cells and/or cell clusters, as well as the approximate number and condition of the cells.

(3) Divide the effusion fluid specimen evenly into two 50 ml Falcon tubes, centrifuge at 400×g for 3 minutes, and discard the supernatant.

(4) After centrifugation, if the cell pellet from a bloody pleural and abdominal effusion appears red, it requires red blood cell lysis. At this point, add red blood cell lysis solution (refer to the reagent kit manual for amount needed) to resuspend the cells, and perform lysis on ice (refer to the reagent kit manual for incubation time). Centrifuge at 4°C at 400×g for 10 minutes, discard the supernatant, and collect the cell pellet. If the cell pellet remains red, repeat the lysis steps mentioned above.

Note: do not lyse excessively, as each lysis process is harmful to all cells; excessive processing will also extend the time of cell exposure to an unsuitable environment, which is not conducive to the subsequent cultivation of organoids.

(5) Add 10 ml of DPBS-PS to resuspend the cells, then transfer to a new 50 ml Falcon tube. Gently mix the cell suspension, centrifuge at 400×g for 3 minutes, and discard the supernatant. Repeat this washing step 2–3 times.

(6) Add 2 ml of 2°C~8°C pre-chilled DPBS-PS to resuspend the cells, filter the cell suspension with a 100 μM cell sieve, and transfer the collected filtrate into a new 50 ml Falcon tube. Centrifuge at 400×g for 5 minutes and discard the supernatant.

(7) Based on the approximate amount of cells and cell clusters obtained from the preliminary microscopic examination, as well as the volume of the cell precipitate harvested in the previous step, add 1- 5 ml of 2°C~8°C pre-chilled DPBS-PS to resuspend the cells.

(8) Cell counting: same procedures as step 2 (10).

(9) Calculate the total number of harvested viable cells and cell clusters, and then adjust

concentration to 4000 to 6000 cells/μl with the complete culture medium supplemented with matrix gel at 1:2 ratio.

Note: This step needs to be kept at 2°C-8°C throughout the process.

Consensus VII: Requirements for Tumor Organoids Cultivation

1.Resuspend the cell pellet with 200 μl of pre-chilled matrix gel (such as Matrigel).

2.Add 50 μl cell suspension to the center well of the pre-warmed 24-well plate.

3.Place the culture plate in a 37°C incubator for 5 minutes. After 5 minutes, invert the culture plate and keep for 25 minutes to allow the matrix gel to solidify.

4.After the matrix gel has solidified, add 500 μl of pre-warmed tumor organoid culture medium at 37°C to the center well. Add 500 μl of DPBS-PS to the surrounding wells to maintain the humidity of the culture plate. Place the culture plate upright in the incubator and culture at 37°C with 5% CO2.

Note 1: It is important to use the appropriate culture medium optimized for the tumor microenvironment of the respective tissue type *in vivo* for the cultivation of tumor organoids from different tissue sources. For example, liver cancer tissue should be cultivated using a specific liver cancer organoid culture medium, which enables the organoids to grow faster and maintains the tissue structure more similar to the source.

Note 2: It is necessary to change the medium once every 2-4 days. Observe the growth of the organoids every day and record photos until passage. Generally, organoids (this is the first generation, i.e., P0) can be observed on the 1st to 3rd day after inoculation.

Note 3: When cultivating primary tumor organoids, the number of cells and cell clusters used should not be too sparse or too dense during inoculation, otherwise the growth of organoids will not be optimal. The appropriate density is shown in Figure 1.

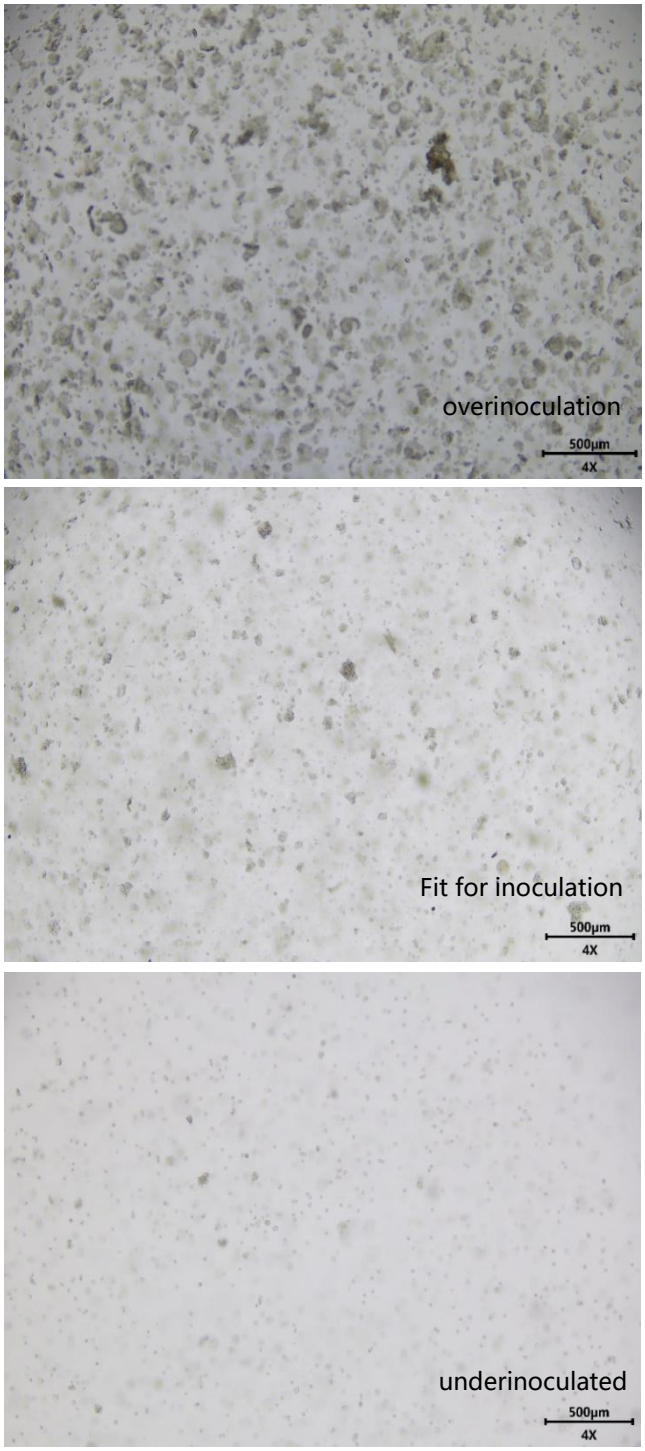

Figure 1. Schematic of Tumor Organoid Culture Seeding Density
（*: From top to bottom, the seeding densities are too high, optimal, and too low, respectively.）

Consensus VIII: Requirements for Tumor Organoid Passage and Amplification

1. Aspirate the culture medium and add digestion solution (refer to the kit manual for the volume). Use a 1 ml pipette tip to aspirate the suspension, pipetting up and down 10 times to disperse the

organoids. Tilt the culture plate to a 45-degree angle and continue to pipette vigorously 10 more times to completely disrupt and dissolve the droplets, and release the organoids.

2. Digest at room temperature (refer to kit manual for digestion time) to disperse the organoids and release the cells. For large and compact organoids, it is advisable to digest at 37°C.

3. Tilt the culture plate at 45 degrees and continue to pipette vigorously 10 to 20 times, transfer the cells into a Falcon tube containing 6 ml of pre-chilled DPBS-PS at 4°C.

4. Take 1 ml of pre-chilled 4°C DPBS-PS to rinse the central culture well and transfer it to the Falcon tube in step 3.

5. Centrifuge at 400×g, 2°C to 8°C for 5 minutes, discard the supernatant, and resuspend the cell pellet in 1 ml of DPBS-PS.

6. Cell counting: Take a small amount of cell suspension and use an automatic cell counter to count the number of cells and cell clusters per ml of cell suspension. Alternatively, count manually with a hemocytometer to calculate the cell concentration under the microscope.

7. Centrifuge at 400×g, 2°C to 8°C for 5 minutes and collect the cell pellet (P0 generation organoids).

8. Resuspend the cell pellet obtained in step 7 with matrix gel at the optimized cell concentration. Generally, the cell number and volume amounts are 5×10^4 cells/25 μl/droplet or 1×10^5 cells/50 μl/droplet.

9. Transfer 50 μl per well to the center of the pre-warmed 24-well plate at 37°C.

10. Place the culture plate in a 37°C incubator for 5 minutes, then invert the plate and continue to stand for 25 minutes until the matrix gel solidifies.

11. Add 500 μl of pre-warmed tumor organoids culture medium at 37°C to each well, and add 500 μl of DPBS-PS to the remaining wells to maintain humidity.

12. Place the culture plate upright and incubate at 37°C with 5% CO2. Change the culture medium every 2 days to 4 days, observe the growth of the organoids every day, and record photos until the required quantity is reached (P1).

Note1：If the P0 generation of tumor organoids is not sufficient to complete the drug sensitivity testing, then it is necessary to perform passage and amplification to increase the quantity. If the quantity of P0 generation tumor organoids is sufficient, the operation of passage and amplification can be omitted and one can directly proceed to the drug sensitivity plate seeding. The decision standard for whether the P0 generation tumor organoids need to be passaged or

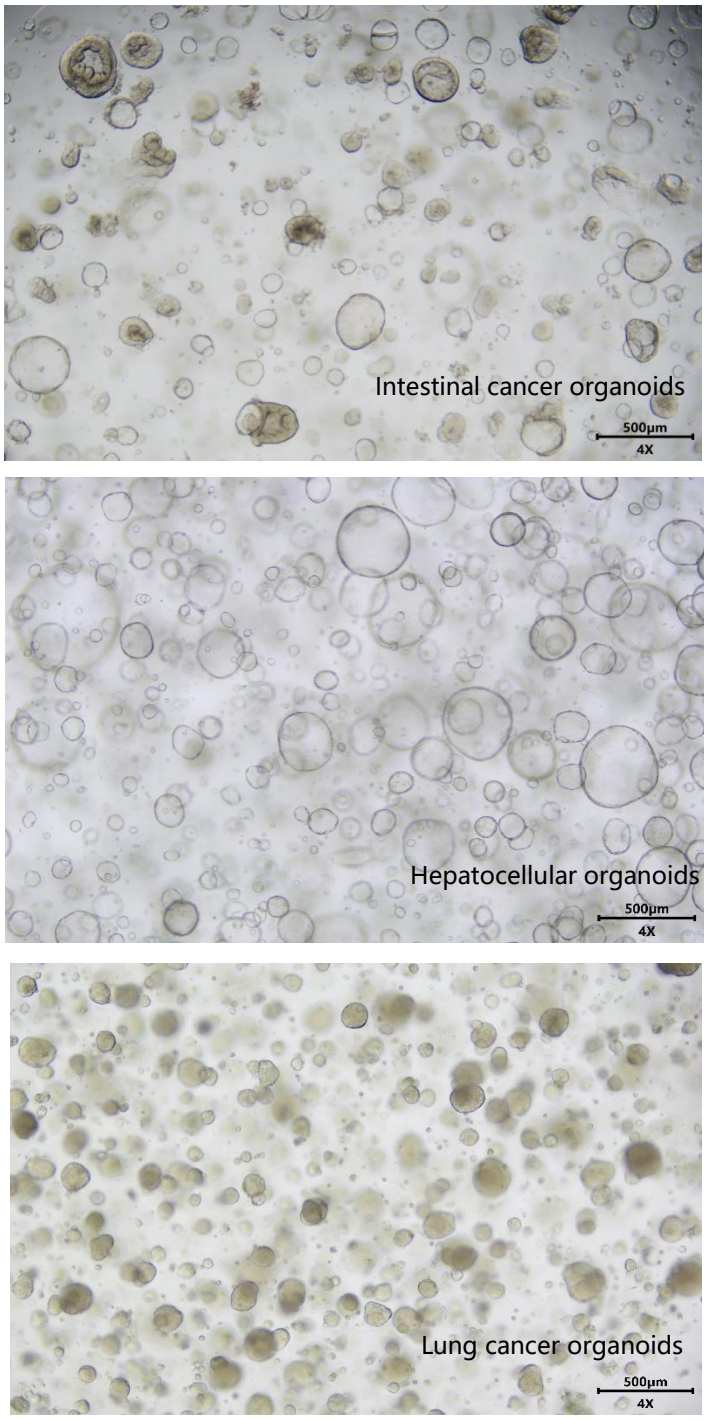

Figure 2. Growth Status of Organoids Before Passaging or Drug Sensitivity Assays

not is that the majority of the organoids should have reached a diameter ≥100 μm.

Note 2: The growth status of organoids that require passage or drug sensitivity plating is shown in Figure 2..

Note 3：Different tumor organoids grow at different rates in their respective culture medium, and organoids from the same type of tumor tissue derived from different patients also vary in growth rate. The growth rate can also be different with the use of organoid culture medium from different commercial sources. Generally, the P0 generation of tumor organoids will reach the density required for drug sensitivity plate seeding or the first passage between 3 to 14 days of cultivation; otherwise, they may undergo apoptosis or necrosis due to insufficient nutrients in the culture medium.

The usual cultivation time for common clinical patient-derived tumor tissue organoids is shown in Table 2:

Table 2. Cultivation Time for Common Patient-Derived Tumor Tissue Organoids

Tumor tissue	Median Time for P0 Organoids to Appear (days)	Median time for P0 generation cultivation to require drug addition or passage (days)
lung cancer tissue	2	10
liver cancer tissue	2.5	14
colorectal cancer tissue	1	7.5
gastric cancer tissue	2	8.5
breast cancer tissue	1.5	8
cervical cancer tissue	2	11
thyroid cancer tissue	1	8
bile duct cancer tissue	1.5	11
pancreatic cancer tissue	2	5.5
ovarian cancer tissue	1	6
esophageal cancer tissue	1	8
lung cancer pleural effusion	1	6
colorectal cancer pleural effusion	1	7
liver cancer pleural effusion	1	9.5
pancreatic cancer pleural effusion	1	3
ovarian cancer pleural effusion	1	3

Consensus IX: Requirements for Drug Sensitivity Plate Culturing and Assay

1.Organoid Digestion

(1) Aspirate the culture medium and add digestion solution (refer to the kit manual for the volume), disperse the gel drop, and digest at 37°C (refer to the kit manual for digestion time). Observe under the microscope to ensure the organoids are digested into uniform cell clusters containing 3 cells-5 cells, then add 1.5 times the volume of DPBS to terminate the digestion.

(2) Transfer the cells into a Falcon tube containing 4°C pre-chilled DPBS-PS, centrifuge at 400×g for 5 minutes, and discard the supernatant.

(3) Resuspend the cells with 1 ml of DPBS, take 20 μl of cell suspension and mix with 20 μl of AOPI staining solution.

(4) Take 20 μl of the stained cell suspension and fill the counting chamber of the cell counting plate, and count with a cell counter.

(5) Top up the Falcon tube in step (3) with DPBS to 4 ml, centrifuge at 400×g for 5 minutes, and discard the supernatant.

2.Organoids Inoculation

(1) Based on the type and quantity of test drugs required in the physician's application form, calculate the number of wells needed for the plate.

Note 1: The number of wells needed for drug sensitivity testing is calculated as 2×a+b, where a is the number of drug treatment regimens agreed upon with the clinic, and b is the number of control wells for the organoids derived from the tumor tissue. For example, if there are 10 drug treatment regimens, the required number of wells is 2×10+4=24.

Note 2: It is recommended to use commercial standard testing drugs for drug sensitivity experiments. Precursor drugs, physicochemical properties of raw materials, and excipients in approved drugs can significantly affect the results of drug sensitivity experiments *in vitro*.

Note 3: Each drug therapeutic regimen can involve a single drug or a combination of two or more drugs. If multiple drugs are added to the same culture well, potential incompatibilities between different drugs *in vitro* should be considered. If necessary, conduct *in vitro* compatibility tests or consult with a clinical pharmacologist before proceeding with experimental operations.

(2) Based on the number of wells needed for the plate, calculate the amount of culture medium and corresponding matrix gel needed for resuspending the organoid pellet. The ratio of culture medium to matrix gel at this time is 2:3.

(3) Resuspend the organoid pellet with the calculated volume of organoid culture medium, then transfer the resuspended liquid to the corresponding amount of matrix gel and mix gently to obtain a cell-containing mixed gel.

(4) Use a single-channel electric pipette to dispense the mixed gel into the drug sensitivity plate (i.e., 384-well plate) at 10μL/well, with a cell content of 1000-2000 cells per well.

(5) Place the 384-well plate in the incubator for 30 minutes, and after removal from incubator add 45μl of appropriate organoid culture medium corresponding to the cancer type to each well.

(6) Record the state of the cells after plating under the microscope.

(7) Continue to incubate the plate in a 37°C 5% CO2 cell incubator.

Note 1: Before inoculation, a plate layout should be designed in advance to determine the experimental groups, the number of wells, and the allocation of positions on the 384-well plate. The requirements for plate layout are shown in Table 3.

Table 3. Requirements and Explanations for Tumor Organoids Drug Sensitivity Plate Layout

Group	Mixed gel	Culture medium	Test drug	Function
Vehicle Group	-	45μL/well	-	(1) Each 384 well plate should have 6 blank wells without organoids.(2) The setting of Vehicle Group aims to eliminate the interference of background fluorescence that may be generated by the culture medium and culture plate on the detection results.
Test Organoid Control Group	10 μL/well	45μL/well	-	(1) 6 replicates per plate; (2) Used to exclude abnormal situations of the test tumor organoids caused by various accidents during the culture period.
Test Organoids Group	10 μL/well	45 μL/well	5 μL/well	(1) According to the drug instructions, chose the lower limit concentration of each effective concentration range for the experiment and set up dual well parallel detection. The mean of the detected values is the final reported value; (2) Noted that the effective drug concentrations listed usually refer to blood concentrations. The actual concentration achievable in the tumor tissue must be adjusted based on factors such as the tumor's location, local blood supply, drug permeability, and the method of administration; (3) Used to obtain drug sensitivity result for the test tumor organoids.

Note 2: When inoculating organoids, ensure that the mixed gel is kept below 4°C to prevent the gel from solidifying.

3.Drug addition

(1) Record the growth status of organoids under the microscope before adding drugs.

(2) Take out the drug stock solution from the -80°C freezer and thaw at room temperature.

(3) Prepare the drug dilution solution: Remove the organoid culture medium such as Advanced

DMEM/F12 from the refrigerator, and add 100 μl of HEPES for every 10 ml of culture medium.

(4) Prepare the working solution of the drug: On the plate for drug preparation, dilute the drug stock solution with the above-mentioned drug dilution solution to a 10× working solution.

(5) Add the working solution of the corresponding drug to the experimental group in the 384-well culture plate, 5 μl/well.

(6) After adding the drug, place the 384-well culture plate in the cell culture incubator and continue to incubate at 37°C, 5% CO2 for 48 hours-96 hours.

Note 1: Take one photo with 4× and one with 20× objective lens.

Note 2: The timing of drug application to tumor organoids from different patients should be determined based on the actual growth status of the organoids after the drug sensitivity plate is laid out. Generally, drugs may be added 24-72 hours after regular culture, when most organoid spheres have grown to about 20-30 μm. In special cases, such as when organoids are digested into single cells for plating, the incubation time after plating should be extended; if the organoids grow faster, drug addition may be done after 24 hours.

Note 3: When adding drugs, change the pipette tip between different drugs and try to avoid creating bubbles in the culture wells.

Note 4: Observe the changes in the number and morphology of organoids after drug application under the microscope every day to determine the time to terminate the culture.

Note 5: After 48-72 hours of drug application, obvious changes in the number and morphology of organoids can be observed under the microscope, such as a decrease in the number of organoids, a reduction in volume, loss of cell luster, dispersion of internal structure, and rupture of cell membranes. If such changes in organoids morphology are observed under the microscope, the culture should be terminated at 72 hours after drug addition; if no obvious changes are observed in daily examination, the culture shall be extended to 96 hours before termination.

4. Requirements for Organoid Viability Assay (Intracellular ATP Activity Fluorescence Detection Method)

(1) Before the viability assay, record the status of organoids under the microscope before the

termination of drug treatment. Take one photo at 4× and one at 20× for both the control group and drug treatment group..

(2) Take out the drug sensitivity culture plate, equilibrate to room temperature for 10 minutes, and add 45 μl of cell viability assay reagent to each well of the blank group, control group, and test group.

(3) Place the culture plate on a plate shaker, shake at room temperature (25°C) in the dark for 3 minutes to promote cell lysis; then rest the plate at room temperature for 15 minutes to stabilize the luminescence signal.

(4) Use a multifunctional microplate reader to measure the luminescence signals in the corresponding wells of the culture plate.

Note 1: The luminescent reagent should be stored at -20°C and must be thawed in cold water or at room temperature before use. It can be aliquoted to avoid repeated freezing and thawing.

Note 2: Aliquoted luminescent reagent tubes opened should not be frozen and thawed more than 5 times.

Note 3: The luminescent reagent needs to be stored and used in the dark.

Note 4: Other than the intracellular ATP activity fluorescence detection method, other methods may also be used to evaluate drug sensitivity test result, such as live-cell staining microscopy, computer-aided tomography, and artificial intelligence (AI) facilitated counting and morphological analysis.

Consensus X: Requirements for Analysis of Results

The intracellular ATP fluorescence intensity data obtained from the multifunctional microplate reader shall be processed using Excel and Graphpad software. The relevant calculation formulas are as follows:

Cell viability value = (OD of the test data - OD of the blank group) / (OD of the negative control group - OD of the blank group) × 100%;

Inhibition rate = [1 - (OD of the drug group - OD of the blank group) / (OD of the negative control group - OD of the blank group)] × 100%;

Average (AVERAGE): is the average value of the detection values within a range;

Standard deviation (STDEVP): is the standard deviation of the detection values within a range;

Z value = 1 - 3 × (Standard deviation of the OD value of the negative control group + Standard deviation of the OD value of the blank group) / (Average value of the OD of the negative control group - Average value of the OD of the Vehicle Group)

Data processing steps are as follows:

(1) Calculate the average value, standard deviation, and Z value of the OD values of the negative control group and the blank group.

(2) Each drug regimen has duplicate wells, and the average cell viability value shall be the mean value of the duplicates.

(3) Calculate the inhibition rate corresponding to each drug regimen.

(4) Store the analysis results in the specified folder.

Note: In the test results, the OD value of the negative control group should be ≥1000 (there may be differences between different instruments), and the ratio of the OD value of the negative control group to the blank group should be ≥10.

Consensus XI: Requirements for Report

The tumor organoid drug sensitivity testing report shall include key information such as patient's name, department, bed number, admission number or ID number, clinical diagnosis, specimen type, specimen collection time, applying physician's name, *etc*., as well as the name and address of the testing laboratory, technical platform, specimen reception time, testing time, report content, report time, organoid culture technician, drug sensitivity testing technician, report reviewing physician, laboratory address, and contact information. The report content should include the name and concentration of each treatment regimen (single drug or combination therapy), its cytotoxic effect or inhibition rate against the tumor organoids, and the laboratory's clinical treatment recommendations based on these test results.

This consensus uses a single concentration at the lower limit of the effective blood drug concentration for drug sensitivity testing. The current recommendations are as follows: an inhibition rate of ≥70% indicates a very strong cytotoxic effect of the drug against the tumor, and

it is recommended as a priority for clinical use; an inhibition rate of <70% and ≥50% indicates a cytotoxic effect, and the drug is recommended for consideration based on the clinical situation; an inhibition rate of <50% and ≥30% indicates a partial cytotoxic effect, and cautious clinical use is advised; an inhibition rate of <30% suggests a weak or absent cytotoxic effect, and clinical use is not recommended—alternative treatments should be considered. Generally, clinicians should prioritize drugs or combinations with high inhibition rates while also considering factors such as drug side effects, the patient's clinical presentation and physical tolerance.

Where applicable, the report may also include an analysis of the specimen quality, photos of the organoids before and after drug administration, *etc*.

Attachments: Abbreviation Index

Abbreviation	Full name	Remarks
TrypLE	TrypLE express Enzyme （1×）	A mild digestion solution for organoids/cells
P0	passage number	The number of cell culture passages, P0 represents primary cells, P1 represents the first generation of cells
DPBS-PS	Dulbecco's Phosphate-Buffered Saline-PS	Dulbecco's phosphate-buffered saline with double antibiotics, used to inhibit bacterial growth and enhance the ability of the specimen to resist bacteria
DPBS	Dulbecco's Phosphate-Buffered Saline	Dulbecco's phosphate-buffered saline
-	CellTiter-Glo Luminescent Cell Viability Assay	A method for quantitative detection of cell viability by measuring the luminescence value of cells
-	Advanced DMEM/F12 with HEPES	Liquid for diluting anti-cancer drugs
-	organoids	organoids
PDTO	Patient derived tumor organoids	Patient derived tumor organoids for testing of drug sensitivity
-	Tumor organoid drug sensitivity testing	Assay performed on tumor organoids to determine drug sensitivity
-	Matrigel	A gel, liquid at low temperature, semi-solid at 37℃, providing spatial support for organoid culture

Expert consensus writing group on patient-derived tumor organoids culture and drug sensitivity detection technology

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Conflict of Interest: All authors declare no conflict of interest

References

1.John Maquart, BA; Emerson Y, Chen.,MD; Vinary Prassad, MD, MPH. Estimation of the Percentage of Patients With Cancer Who Benefit From Genome-Driven Oncology. JAMA Oncol. 2018;4(8):1093-1098.

2.Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 2018 Feb 23；359(6378):920-926

3.Jarno Drost1, Hans Clevers. Organoids in cancer research. Nat Rev Cancer.2018;18(7):407-418.

4.Georgios Vlachogiannis, Somaieh Hedayat, Alexandra Vatsiou, et al. Patient-derived organoids model treatment response of mrtastatic gasetrointestinal cancers. Science. 2018：359（6378）:920-926.

5.Han-Min Wang, Chan-Yuan Zhang, Kai-Cheng Peng, et al. Using patient-derived organoids to predict locally advanced or metastatic lung cancer tumor response: A real-world study. Cell Reports Medicine 4, 100911，February 21，2023，https://doi.org/10.1016/j.xcrm.2022.100911

6.Ming Zu, Xinyu Hao, Jing Ning, et al. Patient-derived organoid culture of gastric cancer for disease modeling and drug sensitivity testing. Biomedicine & Pharmacotherapy ，2023:163,114751

7.Tomayko M M, Reynolds C P. Determination of subcutaneous tumor size in athymic (nude) mice[J]. Cancer Chemotherapy and Pharmacology, 1989, 24(3): 148-154.