Chapter 5: Sterility, Contamination, and Farmer-Level Lab Discipline

In the last two chapters, you built the idea of a small culture space and learned the basic tools, containers, and materials. Now we come to the habit that makes all of those things useful: sterile discipline.

Plant tissue culture is not only about making plants grow. It is also about stopping other living things from growing first.

A tissue culture jar usually contains water, minerals, sugar, vitamins, and sometimes plant growth regulators. This is good food for a tiny plant. It is also good food for bacteria, fungi, yeasts, and algae. A banana shoot tip may need weeks to grow. A fungus can cover the same jar in a few days. A bacterium can multiply so fast that a clear medium becomes cloudy before the plant has made one new leaf. Contamination is therefore one of the central technical problems in plant tissue culture, and professional texts treat aseptic technique as a foundation skill, not as an optional detail (George et al., 2008; Smith, 2013).

The main idea of this chapter is simple:

In tissue culture, cleanliness is not a single action. It is a chain of habits that protects every culture from the mother plant to the final rooted plantlet.

What “sterile” really means

In ordinary farm language, “clean” often means that something looks free of mud, dust, or old plant material. In tissue culture, that is not enough.

A surface can look clean and still carry living microorganisms. A scalpel can shine like new and still carry fungal spores. A jar can look dry and empty but still contain bacteria in a small crack or under the lid. A worker’s hands can look clean after washing but still carry microorganisms from skin, clothing, or the air.

A microorganism is a living thing too small to be seen clearly with the naked eye. In tissue culture, the important groups include:

- Bacteria, which are single-celled microorganisms. Some form slimy films or cloudy growth in medium.
- Fungi, including molds, which often grow as threads called mycelium.
- Yeasts, which are fungi that often grow as creamy or cloudy colonies.

- Algae, which may appear as green growth when light, moisture, and nutrients are present.

A contaminant is any unwanted organism or material that enters the culture system. In this chapter, contamination mainly means unwanted microorganisms growing in or on the culture.

The word sterile means free from living microorganisms. In strict science, sterility is an absolute idea: something is sterile or it is not. In practical farm-level tissue culture, you should think of sterility as a goal reached through repeated control steps. You cannot see every microbe, so you build a system that greatly lowers the chance that microbes survive and enter the culture. This is why plant tissue culture laboratories use sterilized media, sterilized tools, cleaned work surfaces, careful air handling, and trained hand movements (Bhojwani and Dantu, 2013; George et al., 2008).

Three related words are important:

Cleaning means removing visible dirt, plant sap, dust, and organic matter. Washing jars with detergent is cleaning.

Disinfection means reducing many microorganisms on a surface, usually with a chemical such as alcohol or diluted bleach. Wiping a work surface with 70% alcohol is disinfection.

Sterilization means destroying or removing all living microorganisms, including resistant forms such as spores. Heating culture medium in a pressure cooker or autoclave is a common sterilization method in tissue culture (Smith, 2013).

These words matter because they describe different levels of control. If a jar still has dried sugar medium inside it, disinfection may not work well because dirt and organic matter can protect microorganisms. First you clean. Then you sterilize or disinfect, depending on the object and purpose.

Aseptic technique: working without adding contamination

The word aseptic means “without contamination.” Aseptic technique is the method of working so that sterile things stay sterile.

This is different from sterilizing. Sterilization is what you do to media, tools, water, and containers. Aseptic technique is how you behave after sterilization.

For example, suppose you sterilize ten jars of medium. If you open one jar near dusty clothing, cough over it, touch the inside of its lid, and leave it open while searching for a label, the jar may become contaminated even though it was sterilized correctly. The sterilization step was good, but the aseptic technique failed.

Aseptic work is built on a few quiet rules:

- Open sterile containers only when needed.
- Keep the open time as short as possible.
- Do not touch sterile surfaces with non-sterile hands or tools.
- Move slowly enough to avoid stirring dust.
- Keep labels, tools, and plant material organized before you begin.
- Separate dirty work from clean work.

In professional laboratories, aseptic transfer is often done in a laminar-flow cabinet, which moves filtered air across the work area. Many farmer-level beginners may start with a still-air box or a carefully cleaned transfer area, but the principle is the same: reduce airborne dust and microorganisms, and keep sterile material exposed for the shortest possible time. Air, tools, hands, explants, and vessels are all common routes for contamination in plant tissue culture (Leifert and Cassells, 2001; Smith, 2013).

Why contamination grows so quickly in culture jars

A plant in the field is surrounded by competitors, weather, sunlight, soil organisms, and natural defenses. A tissue culture explant is different. It is cut, wounded, small, and placed in a protected container with sugar-rich medium.

An explant is the piece of plant used to start a culture. It may be a node, shoot tip, leaf piece, meristem, embryo, or another small plant part. When the explant is cut, plant juices can leak from the wound. These juices contain sugars, minerals, and other compounds. If bacteria or fungi are present, they can use these materials to grow.

Culture medium often contains sucrose, a sugar added because many small explants cannot photosynthesize enough for strong early growth. This helps the plant, but it also helps contaminants. Many bacteria and fungi grow rapidly on nutrient media containing sugar, which is why contamination can become visible soon after culture initiation (George et al., 2008).

A useful way to think about contamination is this:

The plant is a slow guest. The microbe is a fast thief.

A clean banana shoot may take several days before you see new growth. A mold spore may produce visible threads in two or three days. A bacterium may make the medium cloudy before the shoot has changed at all. This does not mean tissue culture is impossible. It means the early steps must be disciplined.

The main sources of contamination

Contamination can enter from many places. Beginners often blame only the air, but air is only one source. A good farmer-level technician learns to look at the whole chain.

The mother plant

The mother plant is the plant from which explants are taken. If the mother plant is dusty, diseased, insect-damaged, or growing in muddy conditions, the explants will carry more microorganisms. Some microbes live on the surface of leaves and stems. Others may live inside plant tissues without obvious symptoms. These internal or hidden contaminants can be difficult to remove by surface sterilization alone (Leifert and Cassells, 2001).

For example, a mint stem taken from a wet, shaded, crowded patch may carry more fungal spores than a mint stem taken from a clean, actively growing stock plant kept off the ground. The second stem is not automatically sterile, but it starts with a lower contamination burden.

The worker

The worker can bring contamination through hands, breath, hair, clothing, phones, notebooks, and careless movement. Tissue culture does not require fear, but it does require self-control.

For example, if you answer a phone during transfer work, then return to handling forceps without re-disinfecting or changing gloves, you have carried contamination from a dirty object into the clean operation. In a tissue culture room, the phone should not be part of aseptic work.

Tools and vessels

Scalpels, forceps, jars, lids, measuring spoons, beakers, and racks can all carry microorganisms. Even if tools were sterilized earlier, they can become contaminated again if placed on a dirty table or touched with unclean hands.

For example, a forceps tip may be flame-sterilized, but if you lay it down on an unsterile cloth, the tip is no longer sterile. In aseptic work, where a tool rests is as important as how it was sterilized.

Culture medium and water

Medium must be sterilized properly because it contains nutrients. Water used for media, rinsing, and stock solutions must also be suitable for the task. Sterile medium can become contaminated if the lid is loose, the vessel cracks, or condensation draws dirty water around the closure.

For example, jars filled too high may boil over during pressure sterilization, leaving sticky medium around the rim. That sticky rim can later support microbial growth and weaken the seal.

Air and dust

Dust carries fungal spores, bacteria, skin cells, and plant particles. Air movement from fans, open windows, sweeping, talking, or fast arm movement can carry dust into open jars.

This is why clean transfer work should not happen immediately after sweeping the room. Sweeping lifts dust. Wet mopping, followed by a settling period, is safer than dry sweeping near culture work.

Clean routines before sterile work

Good aseptic work begins before the first jar is opened.

A useful beginner rule is:

Do dirty work early, clean work later, and sterile work last.

Dirty work includes washing used jars, removing old cultures, cutting plant material in the field, cleaning shelves, and handling waste. Clean work includes preparing labels, arranging sterilized tools, wiping surfaces, and organizing media. Sterile work includes opening vessels, transferring explants, and closing cultures.

If these tasks are mixed together, contamination spreads. If they are separated, the whole system becomes calmer.

A farmer-level daily routine might look like this in practice:

1. Remove waste and old contaminated cultures from the work area.
2. Wash reusable jars and tools away from the transfer area.

3. Wet-mop the floor or clean the room without raising dust.
4. Wash hands and put on clean clothing or a clean apron.
5. Wipe the work table or transfer box.
6. Arrange only the materials needed for one batch.
7. Disinfect outer surfaces of vessels or packets before placing them in the clean work zone.
8. Begin transfer work only when everything is ready.

This is not about making the room look impressive. It is about reducing the number of microorganisms before you begin the delicate step of opening cultures.

Handwashing, gloves, and personal discipline

Hands are never truly sterile in normal farm work. The goal is to lower risk.

Wash hands with soap and clean water before culture work. Dry them with a clean towel or disposable towel. If gloves are used, they should be clean and intact. Gloves do not make careless hands safe. A gloved hand that touches hair, a phone, a door handle, or a dirty bottle is contaminated.

A good habit is to treat gloves as “clean tools,” not as magic protection. If they touch something dirty, disinfect them again or replace them.

Hair should be tied back or covered. Loose sleeves should be avoided. Talking over open jars should be minimized. Breath can carry droplets, and droplets can carry microorganisms. The same is true for coughing and sneezing. If you are sick, especially with coughing or sneezing, do not do transfer work that day if it can be avoided.

The best tissue culture worker is not the fastest worker. The best worker is steady, prepared, and repeatable.

Cleaning the work area

Before aseptic work, the work surface should be cleaned and then disinfected. If there is visible dirt or dried medium, remove it first. Disinfectants work best on surfaces that have already been cleaned because organic material can reduce their effectiveness (Rutala and Weber, 2008).

For a small farm tissue culture area, cleaning should include:

- The transfer table or still-air box.
- The outside of culture vessels before they enter the work area.

- Tool handles.
- Bottle surfaces.
- Racks and trays.
- Nearby shelves that may drop dust.

Many tissue culture workers use 70% alcohol for wiping small surfaces and tools. Alcohol is flammable, so it must be kept away from open flames. If flame sterilization is used, alcohol containers should be closed and placed safely away from the flame.

Diluted bleach solutions are also used for disinfection, especially for surfaces and plant material preparation, but bleach can corrode metals and irritate skin and lungs. Bleach must not be mixed with acids, ammonia, or other cleaners, because dangerous gases can form. General disinfection guidance recognizes both alcohols and chlorine compounds as useful disinfectants when used correctly, while also emphasizing contact time, surface cleaning, and safety precautions (Rutala and Weber, 2008).

Chapter 9 will teach surface sterilization of explants in more detail. For now, remember the principle: disinfectants need correct concentration, enough contact time, and a surface they can reach.

Sterilizing media and containers

Culture medium is usually sterilized with moist heat under pressure. Moist heat is effective because hot steam transfers heat well and damages essential structures in microorganisms. In laboratories, an autoclave is commonly used. At farm scale, a pressure cooker may be used for learning and small batches, if it can safely reach and maintain suitable pressure and if the operator follows the manufacturer’s instructions.

A common laboratory condition for many media is about 121°C under pressure for a suitable holding time, often around 15–20 minutes after the target temperature is reached, though the correct time depends on volume, container size, load arrangement, and equipment performance (Smith, 2013; George et al., 2008). A large bottle of medium heats more slowly than a small jar. A tightly packed pressure cooker heats less evenly than a well-spaced load.

This is important for beginners. Sterilization time is not simply “how long the cooker was on the stove.” The medium must actually reach the required temperature for enough time. If the pressure cooker is overloaded, if air is not vented properly, or if heating is uneven, some containers may not be sterilized.

For farmer-level work, use these practical principles:

- Do not overfill vessels.
- Leave space between containers so steam can circulate.
- Make sure lids are suitable for pressure heating.
- Do not seal vessels so tightly that pressure cannot equalize, unless the vessel system is designed for it.
- Allow pressure to return to normal before opening.
- Let hot media cool safely and undisturbed.
- Label sterilized batches clearly.

If possible, use autoclave tape or another sterilization indicator as a process check. Indicator tape does not prove perfect sterility inside every jar, but it helps show that the outside of the load reached a high-temperature condition. For serious production, equipment validation is more important than guessing.

Sterilizing tools during transfer work

Tools such as scalpels and forceps must be sterile when they touch explants or enter culture vessels.

In many small laboratories, metal tools are sterilized before work by autoclaving or pressure cooking, then kept wrapped until use. During transfer work, tool tips may be re-sterilized using a flame or a glass-bead sterilizer. Flame sterilization can work for metal tips, but it brings fire risk and requires careful cooling before touching plant tissue. A hot scalpel can kill a small shoot tip.

For example, if you flame forceps and immediately pick up a tiny meristem, the heat can damage the plant. A disciplined worker sterilizes the tool, allows it to cool in sterile air or by touching sterile medium away from the explant, and then handles the plant.

If using alcohol and flame, remember that alcohol is flammable. Keep the alcohol container away from the flame. Never flame near an open alcohol bottle. Many beginners make the mistake of combining too many hazards in a small space. Clean work should be calm, not dramatic.

Disposable sterile blades can reduce some risks, but they still become contaminated after use. A sterile blade that touches the outside of a jar, a dirty label, or a worker’s glove is no longer sterile.

Recognizing contamination

A beginner should inspect cultures often, especially during the first week after initiation and after each subculture. Contamination may appear quickly, or it may remain hidden for some time. Some microorganisms grow slowly or stay inside plant tissues before becoming visible later (Leifert and Cassells, 2001).

Learn the common signs.

Bacterial contamination

Bacteria often appear as:

- Cloudiness in liquid around the explant.
- A milky or creamy film on the medium.
- Wet-looking slime near the cut end of the plant.
- Small shiny colonies on the medium surface.
- A bad smell when the vessel is opened.

In solid medium, bacteria may spread as a wet halo from the base of the explant. In liquid medium, they may make the whole medium cloudy.

Example: A cassava node looks green on day 1. By day 3, the base is surrounded by a pale cloudy zone, and the medium looks wet and shiny. This is likely bacterial contamination.

Fungal contamination

Fungi often appear as:

- White, gray, green, black, or pink fuzzy growth.
- Thread-like strands spreading across the medium.
- Round colonies that become powdery as spores form.
- Growth starting from the explant surface or from the jar edge.

Example: A strawberry leaf piece looks clean after transfer. Four days later, a white cottony growth appears at one corner of the medium and spreads upward. This is likely mold contamination.

Yeast contamination

Yeasts may look like:

- Creamy colonies.
- Cloudy patches.

- Small wet dots that slowly expand.
- Sometimes a fermented smell.

Yeast contamination can be confused with bacterial growth. Both can appear wet or creamy. For beginner work, exact identification is less important than correct action: isolate the culture, record it, and do not open it in the clean area.

Algal contamination

Algae may appear as green films or patches, especially where light, moisture, and nutrients are available. Algae are less common inside well-closed sterile culture vessels than bacteria and fungi, but they can occur if water, containers, or closures are contaminated.

What is not contamination?

Not every color change is a microbe.

Plant tissues may release brown compounds after cutting. This is often called browning or phenolic oxidation. Many plants, such as banana, woody species, and some medicinal plants, release phenolic compounds when wounded. These compounds can darken the medium and sometimes harm the explant. Browning is a plant stress response, not necessarily contamination.

Condensation is also not contamination. Water droplets inside a jar can form when warm medium cools or when room temperature changes. Condensation can increase risk if it runs across contaminated surfaces, but droplets alone do not prove microbial growth.

Callus is not contamination. Callus is a mass of plant cells that may form around a wound. It is usually firm or compact, cream, pale green, yellowish, or sometimes brown. Fungal growth, in contrast, often looks fuzzy or thread-like.

The beginner’s question should be: “Is this growth organized plant tissue, or is it spreading like a microorganism?”

What to do when you find contamination

When you find a contaminated culture, do not panic and do not open it in the clean work area.

A contaminated culture is a source of spores and cells. Opening it near clean cultures can spread the problem. The safest simple response is:

1. Mark the vessel as contaminated.
2. Remove it from the culture shelf.
3. Keep it closed.
4. Record the culture code, date, crop, and visible symptoms.
5. Decontaminate or discard it according to your waste routine.

For reusable glass vessels, contaminated contents should be sterilized before washing whenever possible. A pressure cooker or autoclave can be used to decontaminate closed or loosened vessels in a safe manner, depending on the vessel and equipment. Never heat a tightly sealed container that may build dangerous pressure. Follow equipment instructions and use heat-safe containers.

Do not try to “save” heavily contaminated beginner cultures by opening them and cutting out the clean-looking part unless you are trained and have a suitable clean setup. Sometimes professional laboratories rescue valuable material, but for beginner farm work, rescue attempts often spread contamination to many more cultures.

A useful rule is:

One contaminated jar is a loss. One contaminated work session can become a disaster.

Preventing contamination through batch thinking

A batch is a group of cultures prepared at the same time under the same conditions. Batch thinking helps you understand whether contamination came from one plant, one tool, one medium preparation, or one work session.

For example:

- If only one jar from a batch of twenty is contaminated, the problem may be a single explant, jar, or handling mistake.
- If all jars from one mother plant are contaminated, the mother plant or explant cleaning step may be the problem.
- If every jar using one medium batch is contaminated, the medium sterilization or vessel closure may have failed.
- If cultures handled by one worker on one day show high contamination, technique or work-area cleaning may need review.

Without batch records, contamination feels mysterious. With batch records, it becomes a problem you can investigate.

A simple culture code might be:

BN-2407-03-ND-A

This could mean:

- BN = banana
- 2407 = July 2024
- 03 = batch number 3
- ND = node explant
- A = medium version A

The exact code is your choice. The important point is that every jar must tell its story.

Labels are part of sterility

Labels may seem like recordkeeping, not sterility. But poor labeling causes dirty work.

If labels fall off, workers open jars to inspect them. If jars are unclear, batches become mixed. If contaminated jars are not marked, they may remain on the shelf and spread spores when handled. If medium type is unknown, failed cultures cannot be traced.

A good label should include at least:

- Crop or plant name.
- Variety or mother plant code, if known.
- Explant type.
- Medium code.
- Date of culture or subculture.
- Batch number.

Write labels before sterile work begins. Do not pause with open jars while searching for a pen. Use labels and ink that tolerate moisture and handling. Put the label where it can be read without opening the vessel.

The clean-to-dirty direction

One of the best habits in tissue culture is to move from cleaner material to dirtier material, not the reverse.

If you must handle several groups in one session, begin with the cleanest and most valuable cultures. Work later with newly collected field explants, doubtful material, or cultures that may contain contamination. If you touch questionable material, re-sterilize tools and reset the work area before returning to clean cultures.

For example, do not subculture clean banana shoots after opening a suspicious jar with cloudy medium. If the suspicious jar must be examined, do it at the end of the session, away from clean cultures, and then clean the area.

This habit is common in many biological workflows: protect the clean material first, and do not carry risk backward.

Quiet movement and prepared hands

Aseptic work has a physical rhythm.

Fast movement pushes air. Reaching across open jars increases risk. Searching for tools while vessels are open increases exposure time. Touching the inside of lids is a common beginner mistake.

Before opening the first jar, arrange the work area so your hands know where to go:

- Sterile tools on one side.
- Explants in a known position.
- Empty sterile vessels ready.
- Waste container placed safely.
- Labels already written.
- Flame or sterilizer positioned safely, if used.
- Alcohol or disinfectant away from flame.

During transfer, open one vessel at a time if possible. Hold lids so the inside surface does not touch the table. Keep vessel openings angled away from falling dust when practical. Close each vessel promptly after transfer.

This may feel slow at first. With practice, it becomes smooth. Speed comes from preparation, not rushing.

Farmer-level lab discipline

A farmer-level tissue culture space does not need to copy every feature of a large commercial laboratory. But it must copy the discipline.

Farmer-level lab discipline means:

- You clean before contamination becomes visible.
- You label before confusion begins.
- You record failures as carefully as successes.
- You do not hide contaminated cultures.
- You do not sell plantlets from doubtful material.
- You repeat the same method long enough to learn from it.
- You change only one or two things at a time when troubleshooting.

This last point is important. Suppose your basil cultures have 60% contamination. If you change the bleach time, the mother plant source, the medium sterilization time, the jar type, and the worker all in one week, you may improve the result—but you will not know why. Better discipline is to change one major factor, record the result, and learn.

Tissue culture rewards patience. A careful farmer with modest equipment can learn more than a careless worker with expensive equipment.

Keeping cultures safe and traceable

A tissue culture plantlet is not just a plant. It is part of a history.

Its history includes:

- The mother plant.
- The date and place of collection.
- The explant type.
- The surface sterilization method.
- The medium.
- The worker.
- The culture room conditions.
- The number of subcultures.
- Any contamination or abnormal growth observed.
- The rooting and hardening record.

This is called traceability. Traceability means you can follow a plant back through its production history. It matters for quality, disease control, customer trust, and your own learning.

For example, if one batch of pineapple plantlets performs poorly in the nursery, traceability lets you ask: Did they come from one mother plant? One medium batch? One rooting treatment? One worker? One shelf? Without records, you only have guesses.

Professional micropropagation systems depend on records and quality control because contamination, off-types, and disease risks can move through production if they are not detected early (George et al., 2008; Leifert and Cassells, 2001). A small farmer should use the same principle at a smaller scale.

A simple contamination log

Every culture space should have a contamination log. It can be a notebook, spreadsheet, or printed sheet.

Record:

- Date contamination was seen.
- Culture code.
- Crop.
- Explant type.
- Medium code.
- Days after culture or subculture.
- Type of contamination suspected: bacterial, fungal, yeast, algal, unknown.
- Location: from explant, medium edge, lid area, whole medium, unclear.
- Action taken.
- Possible cause.
- Follow-up change, if any.

This log becomes valuable after several batches. You may notice that ginger explants from rainy-season field plants contaminate more than those from clean potted stock plants. You may notice that one jar lid type fails more often. You may discover that contamination rises when the pressure cooker is overloaded.

The log turns failure into information.

When contamination is not your fault—but still your responsibility

Some plant material is naturally difficult. Underground crops such as ginger, turmeric, taro, yam, and garlic often carry more surface and internal microorganisms because they grow in close contact with soil. Woody plants may carry endophytes, which are microorganisms living inside plant tissues. Some endophytes do not cause visible disease in the mother plant but can grow in tissue culture conditions (Leifert and Cassells, 2001).

This means a beginner should not judge skill only by one difficult crop. Mint nodes may be easier than ginger buds. African violet leaves may be easier than field-grown bamboo nodes. Banana shoot tips from clean nursery suckers may behave differently from suckers dug from wet soil.

However, “difficult” does not mean “ignore discipline.” Difficult crops require better mother plant preparation, careful explant choice, adjusted surface sterilization, and sometimes professional support. Chapter 8 and Chapter 9 will build those skills.

The chapter’s practical lesson

Sterility is not a feeling. It is not the smell of alcohol or the shine of a clean table. It is the result of many small correct actions.

A beginner should remember these five principles:

1. Start clean. Choose healthy mother plants and clean the room before transfer work.
2. Sterilize correctly. Media, tools, vessels, and water must be treated according to their purpose.
3. Work aseptically. Keep sterile items sterile by careful movement and short exposure.
4. Recognize contamination early. Remove and record doubtful cultures without opening them in the clean area.
5. Trace every batch. Labels and records turn mistakes into learning.

In the next chapter, you will learn what goes inside the culture vessel: nutrients, sugar, gelling agents, water, and pH. But remember this chapter whenever you prepare medium. A perfect recipe in a contaminated jar is not a tissue culture success. It is only food for microbes.

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