Guide to sterile technique

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Disinfected: cleaned in order to destroy microbes, but not necessarily free from microbes entirely and still contains DNA/RNA (example: a glove sprayed with 70% EtOH)
Sterile: free of living microbes, but may still contain DNA/RNA (example: autoclaved media)
Molecular grade: label found on many laboratory supplies, entirely free from living microbes and free of DNA, RNA, and DNAse/RNAse (example: molecular grade water)

If you use a disinfected tool in a sterile or molecular grade material, you contaminate it because there are microbes and DNA still present. If you use a sterile tool in a molecular grade material, you contaminate it because there is still DNA and RNA present.

About contamination

(from Wikipedia) Accidental introduction of "foreign" material can seriously distort the results of experiments where small samples are used. In cases where the contaminant is a living microorganism, it can often multiply and take over the experiment, especially cultures, and render them useless.
Contamination compromises experiments, so it is especially important to use good sterile technique. This is the most important thing to learn and use in a microbiology lab.

Common sources of contamination:

- **You**: you are full of bacteria: on your skin, in your hair, on your breath. Touching or breathing on your samples/tools will contaminate them
- **Air:** the air has spores and bacteria floating around in it which can fall into your sample or on your tools
- **Unsterilized laboratory equipment:** self-explanatory
- **Other samples** (cross-contamination): if you allow material from one sample into another, such as by using the same spatula or pipette tip, you contaminate the sample with microbes or DNA that are not intended to be there, compromising the experiment

Sterile technique (aka aseptic technique)

Sterile technique is used to keep disinfected, sterile, and molecular grade laboratory supplies free of contaminating microbes and DNA/RNA/proteins. Best practices for sterile technique include:

- wiping your workspace and supplies with 10% bleach or 70% EtOH before getting started
    - allow bleach to sit for 10 min before rinsing with water and wiping with wet cloth
    - allow 70% EtOH to air-dry for maximum effect
- shining UV light on workspace for 30 or more min
- wearing gloves sprayed with 70% EtOH, and changing them frequently or if they become contaminated
- minimizing air exposure
- not breathing on or touching sterile tools or samples (ie hold your breath near exposed samples/tools, don't speak near exposed samples/tools, open microcentrifuge tubes properly to avoid thumb-in-tube)
- proper use of a Bunsen burner near your workspace to create a sterile field
- slow, deliberate, and controlled actions, paying attention to where exposed sterile tools/materials are at all times and what they come into contact with so you can identify when contamination happens
    - this is especially important when working with pipette tips and serological pipette tips, which can easily touch contaminated surfaces if you aren't paying attention to where they are
    - do not touch the body of the pipettor to the walls of sample tubes, only the sterile tip itself
- working in a laminar flow hood or biological safety cabinet (wiped with bleach, ethanol, and/or UV light)

Not all these practices are applicable to every situation or experiment, you need to decide what methods are required and feasible for your work.

Methods to sterilize/disinfect

70% EtOH (disinfection): Ethanol kills microbes by disrupting the membrane and rupturing cells, not all microbes can be killed this way though.
Sodium hypochlorite (10% bleach, sterilization): Bleach kills microbes and destroys DNA by oxidation.
UV light (sterilization): UV light causes the double strands of DNA to dimerize. This causes microorganisms to die if they are exposed to UV light for too long and causes DNA to become un-amplifiable.
Autoclave (sterilization): A pressure chamber that exposes supplies to high temperature steam for a prolonged period, killing all living organisms. Depending on what is being sterilized, differing amounts of times must be used to completely sterilize the material (ie liquid media requires 25 min but a soil sample requires multiple rounds of 1 hr). Autoclaving does not remove DNA, and some microbial species that create spores can survive short rounds of autoclaving.
Filtering (sterilization): A filter that is 0.22 um or smaller will prevent bacteria from flowing through into the sample, effectively sterilizing it. This does not remove DNA or viruses, though. You must use proper sterile technique while filtering.
Flame (sterilization): Heat denatures and oxidizes proteins and other cellular structures, killing microbes and destroying DNA.

When in doubt, do everything you can to prevent contamination using appropriate methods. It is better to take your time and be careful than to compromise months-worth of expensive experimental samples due to negligence.