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Application-Specific Requirements for Consumables in the Laboratory

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Abstract

The demands placed on laboratory consumables made of plastic are constantly increasing. They must withstand high mechanical and thermal loads, have to be resistant to a wide range of chemicals and free of various contaminants. Sterility and biological purity has increasingly become a basic prerequisite for working with consumables. Likewise, the purity of reaction tubes and pipette tips with regard to heavy metals (e.g., cadmium) has been a fundamental requirement for a long time and has been taken into account by many manufacturers. Especially in the fields of biology, pharmacy and medicine, there is a significantly growing need for higher degrees of purity with regard to nucleic acids, degrading enzymes and pyrogens.
This guide summarizes critical points to be considered for routine applications in the lab and provides tips and recommendations for choosing the best suited purity grade for the required consumables.

Working in trace analysis

One of the key challenges in modern analytical chemistry is to minimize any background interference. This requires not only ultrapure chemicals and reagents but also ultraclean sample handling consumables: tubes, tips and plates. Particularly usage of aggressive organics solvents may lead to release (leaching) of unwanted additives or heavy metal ions, which may interfere with sensitive analytical methods [1, 2]. In general, most plastic consumables may be used in inorganic trace analysis. This also holds true for colored tips and micro test tubes. In cases of uncertainty, such consumables should be checked by letting them leach for several hours in strong inorganic acid (except fuming nitric acid and fuming sulfuric acid). In organic analytics (gas chromatography, thin layer chromatography), the additives can exert a disturbing effect if they can be dissolved out of the plastic. Whether this is possible depends on the solvent used and the additives. It is recommended that a blind sample is always analyzed. Directly before use, the tips should be rinsed several times with the pure solvent to eliminate soluble substances that can be found on the surface.

Working with infectious material

Working with infectious material often requires working with valuable samples in limited quantities. In addition to careful handling of the precious sample material to avoid sample loss, it is also important to protect the user as much as possible when handling the infectious material.

Important prerequisites for transferring infectious liquids are:

> Pipetting with positive displacement pipettes or multi-dispensers to hermetically seal the sample within the tip (Figure 1)
> In terms of air-cushion pipettes: sterilized pipette tips with an integrated filter (e.g. ep Dualfilter T.I.P.S.®, ep Dualfilter T.I.P.S.® SealMax)
> Tips that can directly be attached to the device (e.g. from the box)
> A pipetting tool with a tip ejection mechanism to avoid any contact with the used tip
> Autoclavable liquid handling tools

Liquid handling systems work according to two different functional principles: using an air-cushion or by positive displacement. Air-cushion piston-stroke pipettes have air between the liquid in the tip and the piston in the pipette with the air operating like a spring. The use of filter tips increases contamination prevention within the pipette caused by aerosols. In positive displacement instruments, the piston is integrated into the tip and comes into direct contact with the liquid. The sample is hermetically sealed within the tip to ensure an advanced contamination prevention.

Working with radioactive material

When working with radioactive material, the focus is not only on processing the sample but also on protecting the user. The use of positive displacement pipetting systems is recommended when working with radioactive liquids. As the sample is hermetically sealed within the positive displacement tips, it provides increased protection for the user. Using air-cushion pipettes is possible when following certain precautions.
The isotope workplace should contain a set of appropriate liquid handling tools and accessories that remain at the given location. It is recommended that complicated operational procedures first be tested in a »cold« run. This will help to identify inadequacies in the set-up. Contaminated consumables must be collected and disposed of in accordance with the Radiation Protection Ordinance. Commercially available laboratory cleaning agents are to be used for the required radioactive decontamination of the liquid handling devices according to the manufacturer's recommendations and the chemical resistance of the devices.

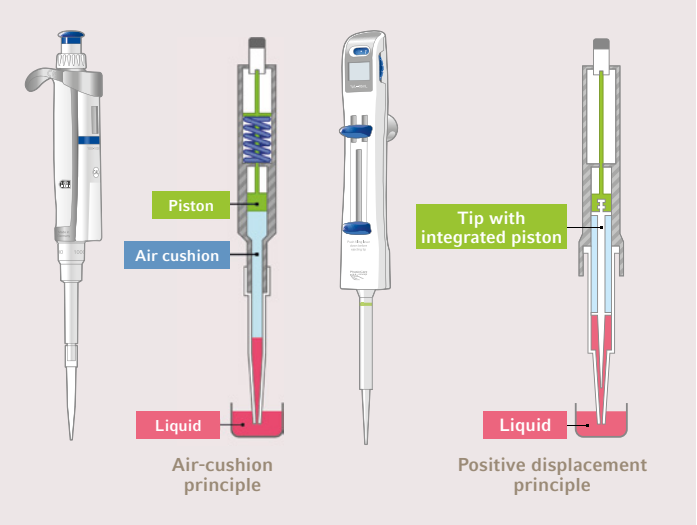

Fig. 1: Air-cushion pipettes and positive displacement systems

Working in the PCR laboratory

Due to the extreme sensitivity of the polymerase chain reaction (PCR), special precautions must be taken in a PCR laboratory to reduce the risk of contamination by native or amplified nucleic acids during test preparation. Ideally, the individual steps, especially sample preparation and processing after amplification, occur in separate rooms or at least seperated work areas with dedicated devices and materials used. Pre- and post-PCR laboratories should each have specific pipettes, tips and disposable tubes that are permanently located there. Pipettes and tip boxes that have once been used for a post-PCR step should never be transferred to the pre-PCR laboratory.
If possible, PCR samples should be pipetted under a closed PCR hood with an integrated UV lamp. If not feasible, a specific workplace with its own set of suitable pipettes should be established which is exclusively used for the preparation of the PCR reaction. Using disposable materials that are free of DNA, DNases and RNases highly decreases the risk of contamination and degradation of nucleic sample material. The preparation of PCR samples with filter tips is recommended to keep the hazard of aerosol contamination as small as possible. The DNA to be amplified should be lastly added to the micro test tube to avoid cross contaminations with other samples and reagents. To avoid contaminations in a PCR laboratory, gloves should always be worn when working and they should be replaced with a new pair at each change between the pre- and post-PCR laboratory. Furthermore, all work places must be cleaned at regular intervals with appropriate cleaning agents to remove DNA residues, DNases, and RNases.

Working in the sterile laboratory

Sterility is a must in cell culture to avoid microbiological contamination. Therefore, all steps have to be performed under sterile conditions. Equipment for sterile work should also be available exclusively for this work area and should not be exchanged between laboratories. The used devices as well as materials have to be sterilized or – if not possible – desinfected thoroughly to remove any potential contamination. In terms of liquid handling tools, many mechanical pipettes are fully autoclavable. When using electronic pipettes, it should be possible to detach the lower part and autoclave it when working in sterile environments.

Several manufacturers offer tips in autoclavable boxes, which have the following characteristics:
> During autoclaving, steam enters the interior of the box
> After autoclaving, the box can be dried in a short period of time

Presterilized consumables are ready to use with the manufacturer's guarantee of sterility. Sterilization occurs through irradiation or gas treatment by specifying the Sterility Assurance Level (SAL), which provides a quantifiable measure of how effective a sterilization process is at reducing the finite probabilty that an organism survives. Autoclaving itself does not necessarily guarantee purity since autoclaving does not remove, but just deactivate contaminations like DNA, RNases or pyrogens.
For this reason, when selecting consumables for sterile work, it is important to ensure that they are not only sterile but also free of other disruptive factors like Pyrogens (e.g., endotoxins), DNA, RNases, DNases, PCR inhibitors.

Purity grades

The constantly growing diversity of requirements in the daily routine of the laboratory requires different purity criteria at a consistently high quality level for consumables. For these purposes, the manufacturer Eppendorf has introduced different purity grades (Tab. 1):
> Eppendorf Quality
> Sterile
> PCR clean
> PCR clean & sterile
> Forensic DNA Grade
> Biopur

Table 1: Eppendorf purity grades for consumables.

	 Eppendorf Quality	 Sterile	 PCR clean	 PCR clean and sterile*	 Forensic DNA Grade*	 Biopur®*
Function, tightness, precision	■	■	■	■	■	■
Low wetting	■	■	■	■	■	■
High chemical resistance	■	■	■	■	■	■
High thermal resistance	■	■	■	■	■	■
High centrifugation stability**	■	■	■	■	■	■
High transparency	■	■	■	■	■	■
Precisely shaped	■	■	■	■	■	■
Lot-specific certified for the following purity criteria						
Human DNA-free			■	■	■	■
DNA-free (human + bacterial DNA)						■
DNase-free			■	■	■	■
RNase-free			■	■	■	■
PCR inhibitor-free			■	■	■	■
ATP-free						■
Pyrogen-free (endotoxin-free)		■		■		■
Sterile (Ph.Eur./USP)		■		■		■
Methods (Examples)						
Applications requiring high general quality, but no checked special purities	■					
Bacteria and yeast cultures		■		■		■
Cell and tissue culture		■		■		■■
Isolation and storage of DNA			■■	■	■■	■
Isolation and storage of RNA			■	■	■	■■
DNA analysis (PCR, restriction analysis, hybridization, sequencing , NGS)			■■	■	■■	■
Mitochondrial DNA analysis					■■	■■
Bacterial DNA analysis						■■
RNA analysis					■	■■
Application Areas (Examples)						
Routine application	■					
Molecular biology			■■	■	■■	■
Microbiology		■		■		■
Cell technology > Stem cell research > Transgenic animals / plants		■		■		■■
Research > Medical research > Agriculture & aquaculture research		■	■	■		■■
Quality control > Food and beverage > Water supply > Environmental monitoring		■	■	■		■■
Forensic			■	■	■■	■

■ Recommended ■■ Highly recommended
* Increased safety due to availability of individually packaged / single-blistered products.
** For accurate details regarding resistance to centrifugation, please refer to the product individual instruction for use.

Quality of Eppendorf consumables

The purity grade "**Eppendorf Quality**" applies of all Eppendorf consumables to guarentee continuous control of quality criteria: function, seal tightness and precision. During production, the consumables are closely and continuously monitored in terms of form, dimensions, appearance as well as essential functions. All production steps right up to product packaging are automated (Fig. 2) to minimize manual interventions and thus contamination risk.

Fig. 2: Fully automatic sorting of Combitips into blister packs

Fig. 3: Packing of consumables with Eppendorf purity grade Biopur prior to sterilization

The purity grade "**PCR clean**" is especially designed for molecular biology analyses to meet the following requirements:

> The consumables must be free of DNases and RNases to protect the very small quantities of genetic material used against those degrading enzymes.
> The consumables must be free of PCR inhibitors that would prevent amplification.
> The consumables must be free of contaminating nucleic acids, as human DNA contamination is one of the main concerns in DNA analysis because it can be passed on to the consumables during the production process.

The purity grade "**Forensic DNA Grade**" meets the requirements of ISO 18385- the standard that specifies requirements for manufacturing products used in collection, storage and analysis of biological material for forensic purposes.

The plastic items used for "**PCR clean**" consumables are manufactured in a controlled environment according to ISO class 8 of ISO 14644-1. The manufacturing areas are spatially separated and only personnel in special protective clothing are granted access. If quality tests are passed, "PCR clean" consumables receive a quality seal which assures their suitability for direct use in molecular biological analysis without the need for further treatment. Each lot with the purity grade PCR clean is tested and certified by an external accredited laboratory to verify the absence of human DNA, DNases, RNases, and PCR inhibitors. In general, lot-specific certificates are available on request and at www.eppendorf.link/certificates.

The purity grade "**Sterile**" meets the expectations for work under aseptic conditions. The consumables are sterilized by irradiation or ethylene oxide gassing in accordance with ISO 11137 or ISO 11135 with a guarenteed SAL of 10^{-6}. During the sterilization transmissible contamination like fungi, bacteria, or viruses are effectively eliminated. Of course, tests are also carried out by an independent, accredited laboratory and the consumables receive the quality seal of sterility accordingly. Lot-specific certificates are provided with sterility testings according to the requirements of European Pharmacopeia (Ph. Eur. 2.6.12) and United States Pharmacopeia (USP).

The quality and purity standard "**Biopur**" was defined to meet the highest demands of the medical, pharmaceutical and food industries in a manner comparable to that of molecular biology and cell technology [3]. It combines the properties of PCR clean and sterile declared consumables. They are also guaranteed to be free of bacterial DNA and ATP. All consumables available in this purity grade are externally tested to be sterile and free of pyrogens, RNases, DNases, DNA and ATP as well as PCR inhibitors. This purity is ensured by an elaborate, automated manufacturing process which safeguards the product against any contamination with biological substances (Fig. 3). These consumables also receive a quality seal and lot-specific certificates tested and issued by an external, accredited laboratory.

Conclusion

The purity of consumables is crucial: it ensures the accuracy and reliability of results in research and the clinic. Any contamination can bias results and thus lead to false conclusions. It is therefore important that all consumables used are of high purity and manufactured under controlled conditions. Maintenance of strict purity standards during their production helps to ensure the integrity of and confidence in scientific results. The consumables and the required purity should therefore be selected based on the application and its challenges, particularly with regard to avoiding contamination.

Literature

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[3] Weiß N. Biopur® - A Unique Dimension in Biological Purity. Eppendorf User Guide 042 (2014).

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