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# $CO_2$ Incubator with Copper Interior: How Effective is Copper-Enriched Stainless Steel, and for Which Internal Parts May Copper Be Recommended?

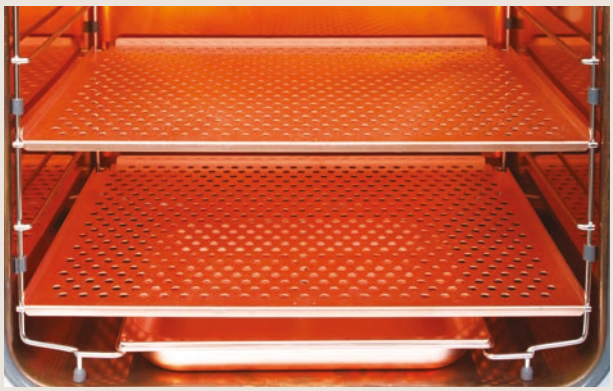
**Natascha Weiß, Wasifa Nurieva**
Eppendorf AG

## Executive Summary

While it is always imperative to prevent contamination in cell culture, copper is a material that possesses strong anti-microbial properties. For this reason, copper, or copper alloy, has been used in the manufacture of $CO_2$ incubators for some time. The proportion of copper within the alloy is crucial for antimicrobial effectiveness.

This paper will demonstrate that a type of copper-enriched steel, which is commonly used in $CO_2$ incubators, does not show significant anti-microbial effectiveness compared with pure copper.

Microorganisms grow and propagate in humid conditions; it is therefore most advantageous to use a water tray, but also shelves (in case of a media spill), with a copper-content that shows antimicrobial effectiveness. Due to significantly higher costs, interior walls made from copper are only recommended if certain properties of the $CO_2$ incubator (such as insufficient tightness of the door seal or inadequate heating of all interior walls) cannot prevent condensation along the walls. A $CO_2$ incubator that is equipped with a copper water tray and shelves is an effective yet economical alternative to a solid copper interior.



## Introduction

**Contamination in cell culture**

In the laboratory, cells are cultivated in $CO_2$ incubators which provide optimized, controlled growth conditions. These include a standard temperature of 37 °C, a relative humidity of up to 95 % and a 5 % $CO_2$ atmosphere to control the pH of the media. Unfortunately, however, these exact conditions also provide ideal growth conditions for mold, bacteria and other microorganisms. If these organisms take hold inside the $CO_2$ incubator, they will pose a high risk of cell culture contamination. This, of course, means that affected cell cultures will have to be discarded, experiments repeated, and the overall reproducibility of results is compromised.

Under normal laboratory conditions, no $CO_2$ incubator can be kept sterile, and especially if used by multiple researchers, repeated door opening throughout the day cannot be avoided. Microorganisms will enter the $CO_2$ incubator via the air, the user or even contaminated flasks or vessels. Microorganisms require moisture in order to propagate. For this reason, the water tray inside a $CO_2$ incubator constitutes the greatest risk factor for contamination, and additives are not a suitable solution. More details on this topic will be discussed below. Furthermore, shelves pose a low, but nevertheless not-to-be-neglected risk as the contact surface for flasks and vessels. Liquid or spilled media which reside on the outside of the vessels may form the basis for microbial growth. The same may be true for the interior wall and doors/door seals of the $CO_2$ incubator if condensation forms due to, for example, uneven heating or leaky door seals.

How can the risk of cell culture contamination inside the $CO_2$ incubator be reduced? The foundation of contamination-free work is based on good aseptic technique and regular cleaning. Additional prevention and decontamination measures exist, which are elaborated and compared in two White Papers [1 White Paper 30, 2 White Paper 50].

Contamination control can also be achieved by employing materials which exhibit antimicrobial properties inside $CO_2$ incubators, where copper, in particular, as well as alloys with a sufficiently high copper content, are capable of markedly reducing germ growth.

**Antimicrobial copper: historic and scientific background**

As far back as antiquity, copper has been used to treat illnesses and wounds, and in the 19th and early 20th centuries, prior to the discovery of antibiotics, copper held a central position in medicine (figure 1) [3]. Today – brought on by increasing bacterial resistance to antibiotics – copper is experiencing a comeback, especially in sensitive areas with high demands on hygiene, such as hospitals. To give an example: a clinical study has shown that if high-touch surfaces (e.g., door handles) in patients' rooms were made of copper, the infection rate was reduced by 58 % compared to those patients whose rooms were equipped with standard materials [4].

Nowadays, multiple scientific publications demonstrate the antimicrobial effects of copper on different bacteria, viruses and fungi such as mold [5, 6]. Pure copper, as well as alloys that contain a minimum of 60 % copper, have been registered with the EPA (US Environmental Protection Agency) as medically effective since 2008 [7].

In addition to the proportion of copper within the alloy, its antimicrobial effect is dependent on several other parameters; for example, this effect is enhanced with increasing temperature as well as humidity [8, 9]. Oxidation of the copper surface, too, has its own unique effect. Exposed to air and over time, copper will develop a natural patina that is brown or green in color (figure 2). Inside the water tray of a $CO_2$ incubator, for example, this oxidation process is accelerated by high temperature disinfection or corroding additives which are not recommended (see below). While this may not look attractive when compared to a shiny and clean copper surface, scientific data prove that the oxidized surface is able to reduce the number of bacteria more effectively than a copper surface that is not, or only slightly, oxidized [8].

This antimicrobial effect, caused by free copper ions on the surface or in liquids, is known as contact killing. Evidence points to a mechanism where first, the cell membrane of the microorganisms is damaged, which subsequently allows the unimpeded influx of copper ions into the cell. This influx leads to the formation of reactive oxygen species which, in turn, will cause oxidative stress. This process is capable of damaging all cellular components (for example, DNA degradation) which will finally trigger cell death [5]. Copper ions, however, do not travel through the air, so that neither copper nor its ions can come into contact with the cells in culture.

**Fig. 1:** Ancient medicinal bronze tool with high copper content

**Fig. 2:** An oxidized copper surface has stronger antimicrobial properties than a shiny copper surface

## $CO_2$ incubators with copper interior

Once the problem "contamination inside the $CO_2$ incubator" and its solution "antimicrobial effects of copper" are brought together, it will be obvious that the answer is to use this material for defined areas within the $CO_2$ incubator. As described in the introduction, the greatest risk stems from microbial growth inside the water tray, and shelves become breeding grounds if media are spilled and not cleaned up immediately. For these reasons, it makes sense to use copper in the production of these exact components. A high-quality $CO_2$ incubator (doors with a tight seal, accurately coordinated heating concept for all surfaces to avoid reaching the dew point) will not permit the formation of condensation under conditions of moderate humidity and sufficiently consistent ambient temperature. If these conditions are not met and, as a result, condensation forms along the inside surfaces, an instrument which is equipped with a copper chamber will be capable of providing enhanced protection.

**Test of the antimicrobial effects of different surface materials inside the $CO_2$ incubator**

In the following test, the antimicrobial effectiveness of copper surfaces (CellXpert® with copper-plated EPA-listed copper material), as well as a copper-enriched stainless steel alloy ($CO_2$ incubator by manufacturer P), compared with a $CO_2$ incubator with stainless steel interior, was evaluated under standard cell culture conditions (37 °C, 85-95 % humidity, 5 % $CO_2$). To this end, the water inside trays made from these three materials, as well as the respective shelves, were inoculated with two test organisms (*Escherichia coli* and *Saccharomyces cerevisiae*). After 6 hours (*E. coli*) and 24 h/48 h (*S. cerevisiae*), samples were taken from the trays and shelves and used to inoculate fresh media. These media were incubated in the shaker, and the resulting turbidity of the culture media was determined via $OD_{600}$ measurements (figure 3). The higher the number of microorganisms capable of replication inside the respective sample, the more turbid the medium and the higher the $OD_{600}$-value.

As shown in figures 4a and 4b, the copper surfaces of the water tray and shelves achieved considerable growth inhibition of both test organisms. In contrast, the copper-enriched stainless steel alloy did not accomplish a significant difference when compared to a stainless steel surface.

These results were verified through simultaneous streaking of the cultures on agar plates; together, they demonstrate that copper surfaces within the $CO_2$ incubator possess antimicrobial effectiveness towards bacteria as well as fungi.

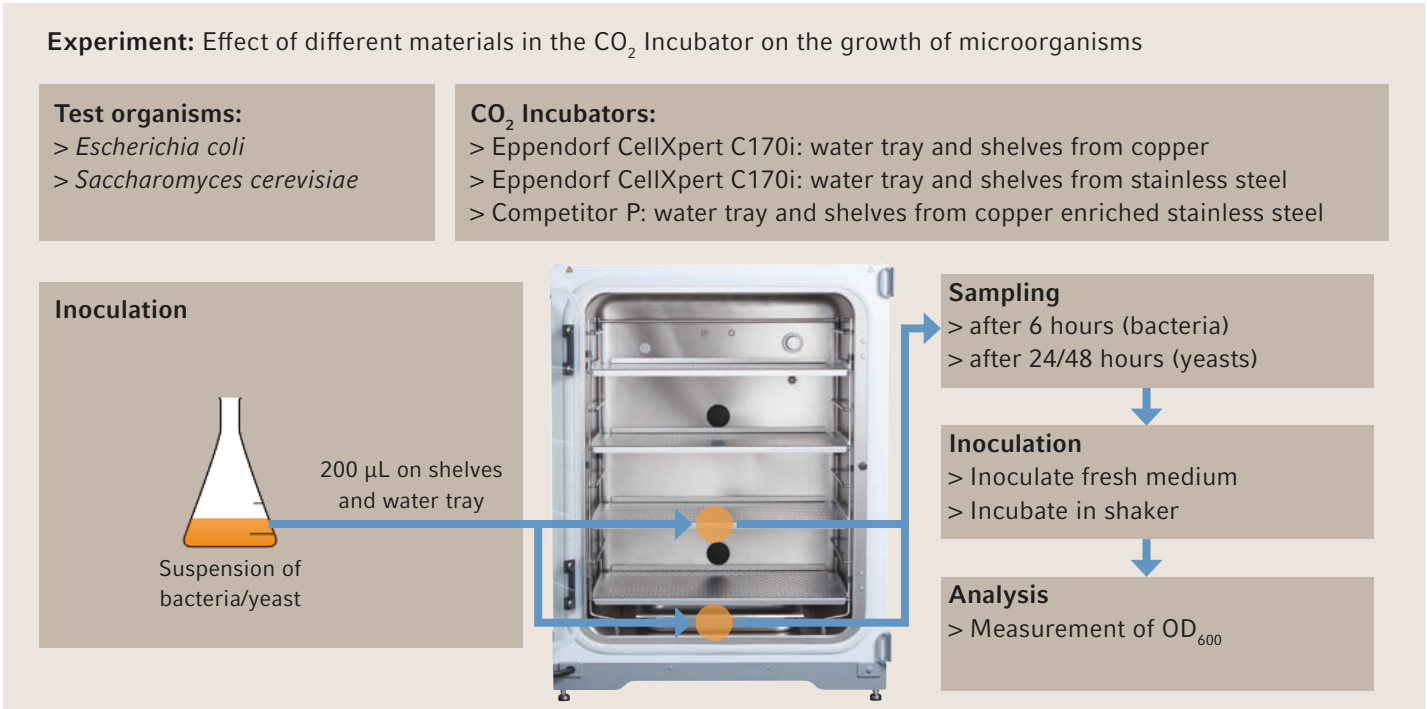



**Fig. 3:** Materials and methods with respect to the test for microorganisms on different surfaces

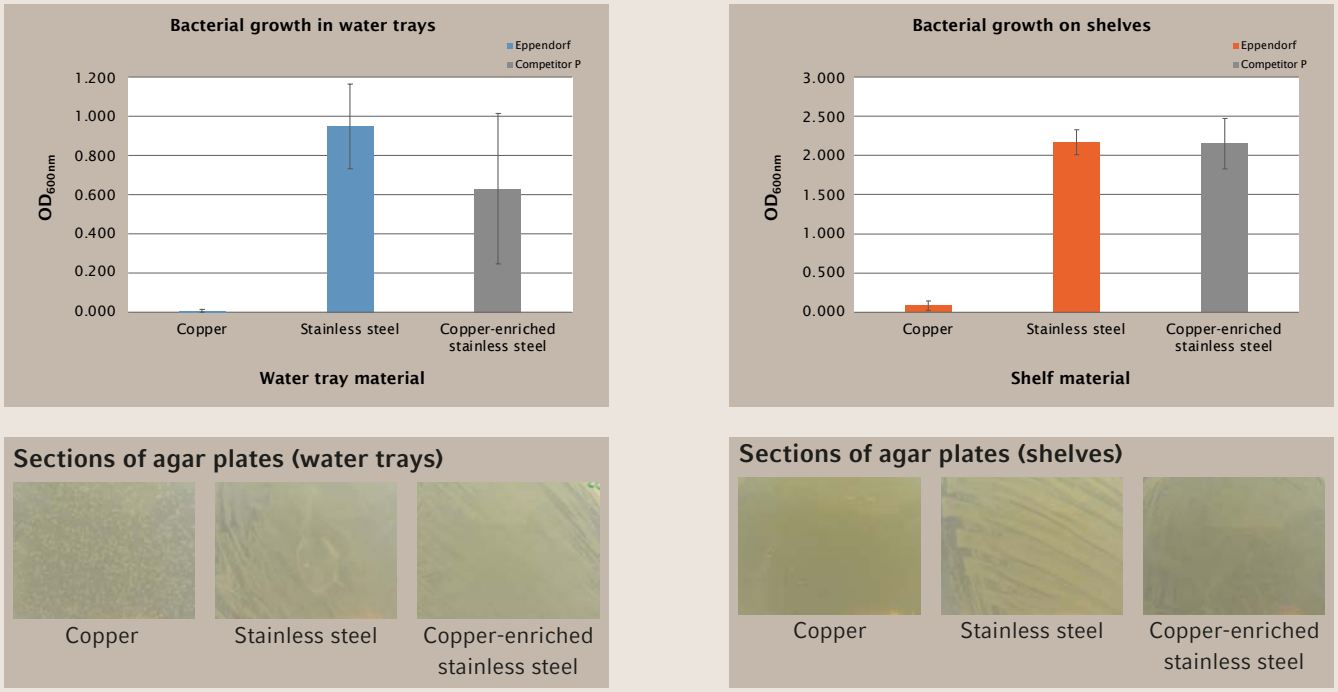



**Fig. 4a:** Results of the growth of *E. coli* on different surfaces in the $CO_2$ incubator


**Fig. 4b:** Results of the growth of *S. cerevisiae* on different surfaces in the $CO_2$ incubator (water trays: after 48 h incubation, shelves: after 24 h incubation)

$CO_2$ incubator water trays and shelves that are made from copper are capable of inhibiting the growth of microorganisms, even under cell culture conditions, and they thus contribute substantially to the prevention of contaminations and their potential spread. In contrast, the respective equipment made from copper-enriched stainless steel did not demonstrate antimicrobial properties within the scope of this experiment.

The main advantage of copper is the fact that its antimicrobial actions are permanent; copper exerts its effects continually and therefore bridges cleaning and decontamination intervals. Furthermore, possible errors, during the cleaning process or when spilled media are not removed right away, will not immediately lead to a massive expansion of microorganisms inside the $CO_2$ incubator.

Since the water tray represents the place of highest contamination risk inside the $CO_2$ incubator, the use of antimicrobial additives to the water is widespread. However, their use should be considered carefully. Additives are potentially corrosive and may damage the tray, especially if they are used incorrectly and the water is not changed on a regular basis. If, on the other hand, the additives contain volatile substances, these will evaporate along with the water and thus potentially impact the cells. Finally, these additives can only (potentially) protect the water tray – they will not protect shelves or interior walls.

Copper, if used throughout the interior of a $CO_2$ incubator, is more expensive than stainless steel. On the other hand, the total costs of contamination may exceed the purchase price many times; additional expenses are incurred through the loss of sample material, the need to repeat experiments, battling the contamination itself and possible downtimes imposed by these measures.

The copper that is used for the copper-plating of the respective components of the CellXpert $CO_2$ incubator is registered with the US EPA as an anti-microbially effective material [7]. Through its natural aging process, it develops a patina which further amplifies the antimicrobial effect of the material. The fact that the water tray and shelves of the CellXpert $CO_2$ incubator are also available separately ensures the availability of an efficient and cost-effective alternative to a $CO_2$ incubator with complete copper interior (chamber + water tray + shelves).

## Conclusion

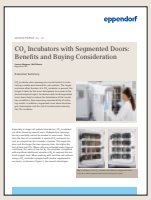
During the cultivation of cells inside the $CO_2$ incubator, the contamination risk must be kept to an absolute minimum. This proves to be particularly challenging if one instrument is used by multiple researchers and frequent opening of doors cannot be avoided. Further to the design of the $CO_2$ incubator, multiple prevention and decontamination measures also play a role [1, 2]. The use of copper surfaces inside the $CO_2$ incubator represents an additional building block within this safety concept through copper's direct and consistent antimicrobial action. It is not necessary for the entire interior to be made from copper; in high-quality instruments which do not harbor an increased risk of condensation, a copper water tray will ensure that this moist location, which is naturally prone to contamination, is protected. Copper shelves offer additional safety in case media are spilled. These components pose a lower cost than a complete copper interior. At the same time, it is imperative to ensure that they are manufactured from the "right" (anti-microbially effective) material. Whereas pure copper is highly effective against microorganisms, this effect decreases with decreasing copper content, and an alloy with insufficient copper content will no longer achieve a significant antimicrobial effect.

### Recommendations for $CO_2$ incubators with copper interior

> Take a closer look at the specific copper material used. What percentage of copper is used in case of copper-enriched stainless steel?
> If an alloy is used: is it listed as antimicrobially effective by the EPA [7]?
> Consider using a copper water tray and shelves, instead of a full copper version including a copper chamber, to save costs and reduce the contamination risk.
> Oxidized copper (e.g. by repeated high temperature disinfection) is more effective than a shiny surface.
> Avoid using water tray additives as these can be corrosive and destroy the water tray. In addition, they might contain volatile substances that can negatively influence your cell cultures and do not provide a protection for your shelves.

## More $CO_2$ Incubators knowledge:

**White paper:** $CO_2$ Incubator Temperature Control: What Is the Best Place For Your Cell Culture Vessels?



**White paper:** $CO_2$ Incubators with Segmented Doors: Benefits and Buying Consideration Segmented door

**Flyer:** How to reduce costs in the cell culture lab with $CO_2$ incubators

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## Literature


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