Lab plasticware and supplies

Thermo Scientific microcentrifuge tubes are free of contaminants

Introduction

Contamination of laboratory reagents with exogenous bacterial genomic material has been previously described to impact the interpretation of microbiota data [1]. The contamination of laboratory consumables, such as buccal swabs, has also been reported [2]. Moreover, it has been recognized that laboratory consumables can contaminate biological and other samples in the laboratory [3]. In response to contamination events, the forensics community recommended guidelines for minimizing the contamination of disposable laboratory plasticware and reagents [4]. Therefore, it is extremely important for manufacturers to minimize the possibility of contamination of laboratory consumables during the production process.

Plastics and consumables from Thermo Fisher Scientific undergo stringent manufacturing standards and are subjected to comprehensive and rigorous testing, assuring that laboratory consumables, such as microcentrifuge tubes, are delivered to customers free of contaminants. Our **Fit for Purpose designations** indicate products that incorporate a variety of testing and quality control procedures in accordance with global standards certified through the International Organization for Standardization (ISO) and United States Pharmacopeia (USP), among others. For example, our products are tested for sterility assurance level (SAL) in accordance with ISO 11137 [5]. In addition to assuring that Thermo Scientific™ products are of exceptional quality and meet or exceed the expectations of scientists, our Fit for Purpose designations provide customers with the ability to easily find and select laboratory consumables ideally suited to specific application requirements [6]. For example, our Thermo Scientific™ microcentrifuge tubes are routinely tested for the presence of human and mouse DNA at <30 pg/µL, RNases at <1 x 10^{-9} Kunitz units/µL, DNases at <1 x 10^{-7} Kunitz units/µL, and pyrogens at <0.5 EU/mL [7]. In addition, our electron beam (E-beam)–sterilized Thermo Scientific microcentrifuge tubes are validated to the ISO 11137 standard, with a SAL of 10^{-6}, and tested for ATP at <1 x 10^{-13} mg/µL [7]. As a result, our microcentrifuge tubes are identified with our Fit for Purpose designations of **PCR ready** and **Sterile & PCR ready**, providing assurance and making it simple to match them for suitable applications.

This report is a review of some of the testing and quality control practices that occur during the manufacturing or post-manufacturing processes of microcentrifuge tubes at Thermo Fisher Scientific. These practices help ensure that our microcentrifuge tubes are free of contamination from human and microbial genetic material and other contaminants.

Post-manufacturing sterilization method for decontamination of human DNA

In many molecular biology applications, particularly in DNA-based forensics workflows, it is especially important to minimize the probability of human DNA (hDNA) contamination. Forensic DNA laboratories rely on manufacturers of tubes and other consumables for a consistent supply of high-quality products with minimal background noise coming from hDNA contamination from manufacturing processes. As a result of going through rigorous testing and quality control processes during manufacturing and sterilization treatment after manufacturing, Thermo Scientific 1.5 mL and 2 mL microcentrifuge tubes can be used in applications and kits where any hDNA contamination must be minimized. The ISO 18385 standard requires a 1,000-fold reduction in hDNA quantity following post-production treatment [8]. This high reduction of contaminant hDNA was confirmed following the post-manufacturing sterilization treatment.

The validation of the post-manufacturing sterilization method included both treated and untreated tubes spiked with 67 ng of hDNA. The tubes were dried in a PCR hood overnight, and half of them went through sterilization treatment with ethylene oxide (EtO). The quantity of DNA from treated and untreated samples was then analyzed by qPCR.

The samples from tubes that went through post-manufacturing sterilization treatment had an average C_t value of 39.9, while those from untreated tubes showed an average C_t value of 26.5 (Figure 1). A difference of 10 in C_t values corresponds to an approximate 1,024-fold difference in DNA quantity [9]. Therefore, the post-manufacturing sterilization procedure of the tubes can reduce hDNA quantity by over 1,000-fold, which is required by ISO 18385 to fulfill hDNA-free label requirements.

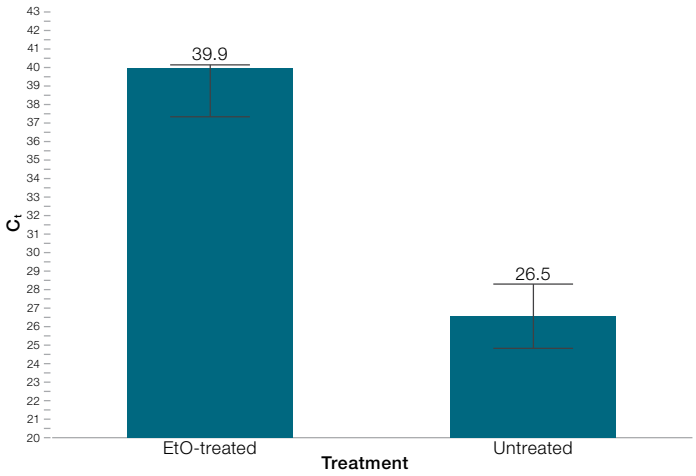

Figure 1. C_t values of hDNA samples from ethylene oxide (EtO)-treated and untreated tubes.

Microcentrifuge tubes are free of human DNA

During the regular manufacturing process, all batches of screw-cap microcentrifuge tubes are tested for the presence of hDNA using endpoint PCR. The testing procedure starts with washing the tubes with sterile PCR-grade water. After washing, the collected water wash is tested for the presence of hDNA by performing PCR with primers specific to human genomic DNA. The negative control is PCR-grade water. In this example, a fraction of the resulting wash was also tested for the presence of PCR inhibitors by spiking it with hDNA prior to PCR. The standard PCR master mix contained *Taq* DNA polymerase, 10X PCR buffer, $MgCl_2$, dNTPs, and human-specific primers in a total reaction volume of 30 µL. PCR reactions were run in a thermal cycler for 30 cycles. 10 µL of the PCR products were then run on a 1.2% agarose gel stained with ethidium bromide, and bands were compared with those of both positive and negative controls (Figure 2). Sample tubes were designated to be free of detectable hDNA only if no 294 bp PCR product appeared in lanes e and f (Figure 2), and there were no bands for negative control samples (lanes a and b). Primers and primer dimers might be visible in the lanes but should not appear as bright as amplified hDNA. Positive controls (lanes c and d) and PCR-inhibition controls (lanes g and h) contained 1 picogram of hDNA, and they confirmed the PCR reaction was well optimized and would produce an amplicon if hDNA was present.

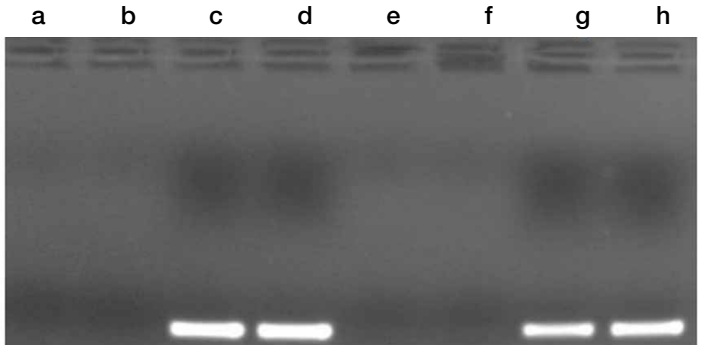

Figure 2. Typical gel results from testing microcentrifuge tubes for the presence of hDNA. PCR products were run on a 1.2% agarose gel stained with ethidium bromide. Shown are negative controls in lanes a and b, positive controls in lanes c and d, tested samples in lanes e and f, and PCR-inhibition controls in lanes g and h.

Thermo Scientific microcentrifuge tubes are free of mycoplasmas and other bacteria

As a part of the quality testing process, Thermo Scientific microcentrifuge tubes are tested for the presence of microorganisms. Samples of tubes selected from production batches are tested by the direct inoculation method wherein water used to wash the tubes is cultured in 100 mL of broth for up to 14 days. Throughout the incubation period, the samples are examined daily for growth of microorganisms, with the first 3–5 days examined for the presence of fast-growing bacteria, the next 5–7 days for molds and yeasts, and the remaining days for slow-growing bacteria.

In addition, the microcentrifuge tubes that go through the post-manufacturing sterilization treatment are confirmed to be free of bacteria after the treatment. For example, after the EtO or E-beam post-manufacturing sterilization procedure is performed, treated microcentrifuge tubes, together with the nonsterilized tubes, are tested for bacterial DNA in an environmentally controlled room according to ISO 8 recommendations [10]. To achieve this, 20 µL of PCR-grade water is added to the tubes, vortexed, and heat shocked at 95°C. 2 µL of this water is then added to a PCR master mix containing bacteria-specific primers. All PCR reactions, including test samples, positive controls, and negative controls, are run in a thermal cycler for 35 cycles. The evaluation of the PCR products is performed on an ethidium bromide–stained 1.0% agarose gel. The success of the assay is evaluated by the presence of bands for the positive controls. Typical gel results are presented in Figure 3.

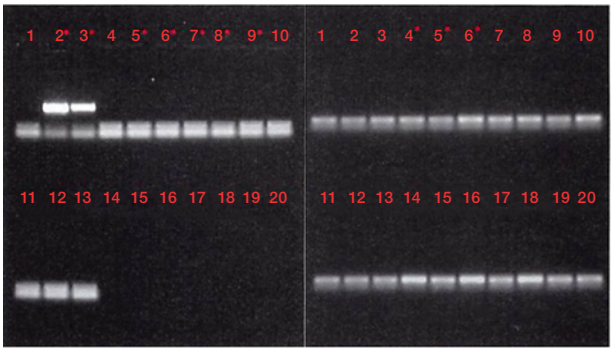

Figure 3. Use of PCR to detect the presence of bacteria. Left gel: nonsterile microcentrifuge tube samples in lanes 1 and 4–13 (only primers visible), positive controls in lanes 2 and 3, and empty lanes 14–20. Right gel: E-beam–treated microcentrifuge tube samples in lanes 1–10, EtO-treated microcentrifuge tube samples in lanes 11–20 (only primers visible).

To confirm the validity of the endpoint PCR test results, microcentrifuge tubes from a separate batch (20 E-beam–treated tubes and 20 untreated tubes) were tested for the presence of bacterial DNA using qPCR. A 20 µL volume of sterile PCR-grade water was dispensed into each tube, and the tubes were vortexed for 5 seconds and centrifuged for 30 seconds. 12 µL of water from each of the tubes was used as a template in the qPCR reaction using the **Applied Biosystems™ TaqMan™ Environmental Master Mix 2.0 (Cat. No. 4396838)** and **Applied Biosystems™ TaqMan™ Microbe Detection Assay (Cat. No. A50137)** per the **quick reference guide** [11]. Also, aliquots of the water were used as templates in qPCR reactions performed with the **Applied Biosystems™ MycoSEQ™ Mycoplasma Detection Kit (Cat. No. 4460623)**, and compared with the provided mycoplasma DNA control. Microbial DNA for positive control reactions was obtained from donor buccal swabs. PCR-grade water was used as a template for negative control reactions.

The qPCR test of both E-beam–treated and nontreated microcentrifuge tubes showed no bacterial presence, and only positive controls (human buccal swabs) showed amplification using the TaqMan Microbe Detection Assay (Figure 4). In addition, there was no presence of mycoplasmas (Figure 5).

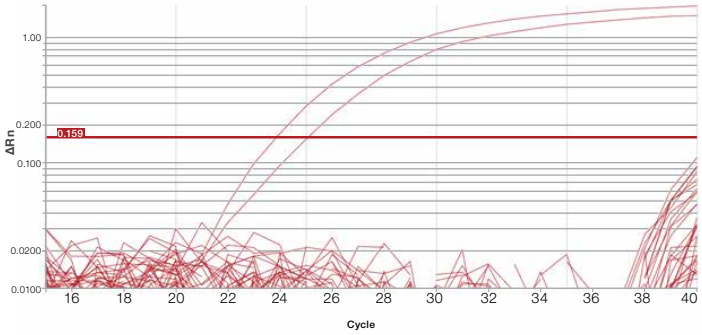

Figure 4. qPCR results from the TaqMan Microbe Detection Assay. All samples from untreated and treated tubes produced no amplicons, with only background noise present below the instrument-set threshold (ΔRn = 0.159), indicating no microbial DNA was in the tubes. The two positive control samples had C_t values of approximately 24 and 25, which demonstrates the assay was functioning as intended.

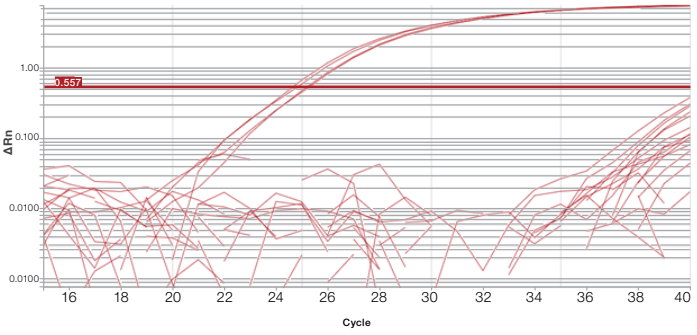

Figure 5. Results from the mycoplasma qPCR assay. Samples from non-sterilized and sterilized microcentrifuge tubes all contained no mycoplasma DNA, demonstrated by background noise below the instrument-set threshold (ΔRn = 0.557) for all reactions. The four positive control samples had C_t values of approximately 25, which confirmed the ability of the assay to amplify mycoplasma DNA.

Thermo Scientific microcentrifuge tubes are free of RNases and DNases

RNases and DNases are in eukaryotic and prokaryotic cells, and they can easily contaminate laboratory spaces and reagents. Because even a minuscule amount of nuclease contamination can lead to degradation of nucleic acid samples and make laboratory experiments inaccurate, it is important to prevent the contamination altogether. RNA is particularly susceptible to cleavage by contaminating RNases, and avoiding contamination is the best way to prevent degradation problems [12]. Thermo Scientific microcentrifuge tubes are tested for RNase and DNase contamination. RNA and DNA are used as substrates in RNase and DNase degradation assays, respectively. Tubes are incubated with RNA or DNA substrate in DNase- and RNase-free water at 37°C for 1 hour, followed by heating to 65°C for 5 minutes. The integrity of the substrate is then assessed by gel electrophoresis. Tubes must exhibit no degradation of either RNA or DNA on 1% agarose gels to be certified RNase- or DNase-free, respectively. The typical results for RNase and DNase testing are presented in Figures 6 and 7, respectively. Lane a (negative control) shows intact RNA from an RNase-free tube, lane b shows the RNA from the tested tube, and lane c shows RNase-degraded RNA (positive control). As indicated by the gel, RNase will degrade RNA samples, which will likely skew the interpretation of results and/or destroy valuable samples. Similar results can be seen for the DNase test; the DNA in the microcentrifuge tube did not exhibit signs of degradation by DNases (Figure 7).

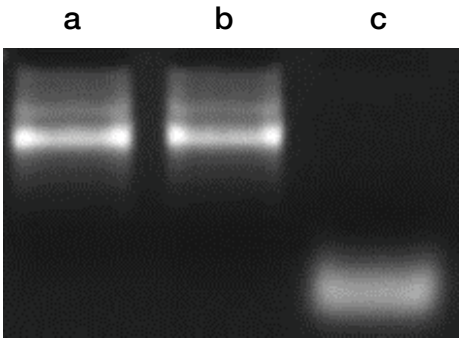

Figure 6. Gel image of the results from testing microcentrifuge tubes for RNase. Shown are the negative RNase control in lane a, tested sample in lane b, and RNA exposed to RNase in lane c (positive RNase control).

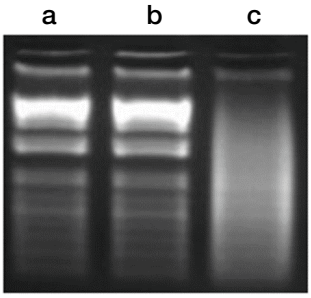

Figure 7. Gel image of the results from testing microcentrifuge tubes for DNase. Shown are the negative DNase control in lane a, tube sample in lane b, and DNA exposed to DNase in lane c (positive DNase control).

Thermo Scientific microcentrifuge tubes are free of mouse DNA

In addition to testing for hDNA, Thermo Scientific microcentrifuge tubes are also tested for mouse DNA. Testing for mouse DNA consists of washing the microcentrifuge tubes with sterile DNA-free water and testing the resulting wash for mouse DNA using endpoint PCR. An aliquot of the water wash is used as a template in the PCR master mix containing mouse-specific primers. For the negative controls, PCR-grade water is used; and for the positive controls, a mouse standard DNA is used as the PCR template. To ensure there is no PCR inhibition, an additional aliquot of the wash from the microcentrifuge tubes is spiked with mouse DNA and amplified in the same PCR run. Following PCR amplification, the PCR products from each experimental sample and the controls are run on an agarose gel and compared. Routine results are shown in Figure 8, with the negative controls (lanes a and b) and the experimental samples from the microcentrifuge tubes (lanes e and f) showing no mouse DNA amplification. The positive controls for mouse DNA (lanes c and d) and the controls for PCR inhibition (lanes g and h) show bands indicating the presence of mouse DNA and the absence of PCR inhibitors, respectively.

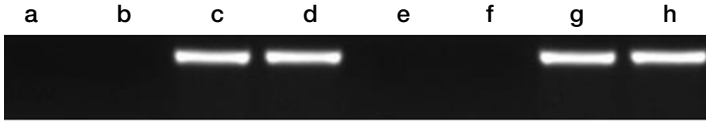

Figure 8. Gel image of results from testing microcentrifuge tubes for mouse DNA using endpoint PCR. Shown are negative controls for mouse DNA (lanes a and b), positive controls for mouse genomic DNA (lanes c and d), samples from microcentrifuge tubes (lanes e and f), and controls for PCR inhibition (lanes g and h).

Thermo Scientific microcentrifuge tubes are free of endotoxins and ATP

Endotoxins are typically any cell-associated bacterial toxins that are part of the outer membrane of the cell wall of gram-negative bacteria. Endotoxins can influence cell growth, cell differentiation, contractility, and protein expression of mammalian cells. During bacterial degradation, endotoxins are released that can reduce transfection efficiency and subsequent protein expression levels. The presence of endotoxins in microcentrifuge tubes and other laboratory consumables can affect *in vivo* and *in vitro* testing results. *In vivo*, the presence of endotoxins can lead to complications such as disseminated intravascular coagulation (DIC), endotoxin shock, and adult respiratory distress syndrome (ARDS). The presence of endotoxins *in vitro* can affect the results and conclusions of experiments. The presence of endotoxins is determined by the limulus amebocyte lysate (LAL) gel clot assay according to the current USP 85 regulation of the FDA [13]. The presence of endotoxins in microcentrifuge tubes is determined regularly by the use of the Thermo Scientific™ Pierce™ Rapid Gel Clot Endotoxin Assay Kit (Cat. No. A43879). A representative sampling of each tube is washed with endotoxin-free water, and wash water is tested at 0.06 EU/mL assay sensitivity for the presence of endotoxins. The endotoxin-free designation is assigned if levels of endotoxin are <0.5 EU/mL.

Adenosine triphosphate (ATP) is a nucleoside that is a source of energy in living cells. The ATP test can detect most cells and other organic contaminants [14] and is used to assure that general hygienic standards are met. Thermo Scientific microcentrifuge tubes are regularly tested for the presence of ATP at $<1 \times 10^{-13}$ mg/µL and found to be free of ATP.

Conclusion

Thermo Fisher Scientific manufactures laboratory consumables under stringent quality standards described in its Fit for Purpose designations and clean claims declaration. Microcentrifuge tubes are delivered to customers with minimal possibility of contamination coming from human, mouse, bacteria, and mycoplasma DNA. In addition, microcentrifuge tubes delivered to customers are free of RNases, DNases, endotoxins, and ATP. Our commitment to supporting scientific discoveries is exemplified by our dedication to manufacturing products of exceptional quality that can meet or exceed customer expectations.

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