Applications
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Technical Report

Contamination Test for Automated Dispensing System - epMotion 5070 and 5075

Renate Fröndt, Eppendorf AG, Hamburg

Introduction

Strictly speaking, the term contamination includes both true contamination and carryover. Carryover is defined as an earlier sample A interfering with a later sample B. Carryover can therefore occur only from A to B and, consequently, carryover is seen only in the transport of liquids and may affect all parts contacting changing liquids. Carryover may be limited to just a single part of an analytical machine or might affect all parts of a flow system.

In contrast to carryover, true contamination is due to external materials entering the sample (splashes of liquid, solid particles, and gases). In the process of contamination, sample A may be affected by sample B and sample B may be affected by sample A.

A variety of qualitative and quantitative methods can be used to determine the degree of contamination. A well-known qualitative procedure in molecular biology involves pipetting PCR reagents into a 96-well or 384-well plate in a chess board-like pattern, in which adjacent wells receive water only, but no sample material (DNA). Once a PCR reaction and subsequent gel electrophoresis are completed, an assessment is made as to whether or not contamination from one well to another occurred.

A quantitative procedure involves pipetting demineralised water and lithium chloride solution (using a saturated lithium chloride solution) in a chess board-pattern into a 96-well or 384-well plate followed by flame photometry to assay for lithium.

This procedure is an accepted method and used for example for testing pipette tips as a means of quality control. This procedure allows a quantitative statement regarding possible contamination levels to be made, since its detection limit is as low as 0.1 nl (= 6.1 ng of Li). According to Specker and Kaiser [1], the detection limit of the measurement of the Li emission by flame photometry is 1 ng Li/ml (=1 ppb Li). A very stable signal is attained from approx. 5 ng Li/ml and higher. Unlike sodium and potassium, which are detectable at similar concentrations, this level can hardly be expected as a coincidental contamination. Consequently, one can be fairly certain that a Li signal in the wells designed to be filled with water only is due to a satellite drop - arising when the Li solution is dispensed or transported to the other wells. To be able to methodically estimate any contamination during pipetting steps , the lithium chloride procedure described above has been used. This method is less susceptible to interference than the biological procedure underlying a PCR reaction. A multitude of interfering factors, ranging from contaminated samples or reagents to erroneous cyclers, etc., can interfere substantially with a PCR reaction. The same interference is not encountered in Lithium chloride pipetting and therefore does not falsify the results obtained.

Materials and Methods

The described method refers to the contamination tests using PCR plates and epMotion 5070/75.

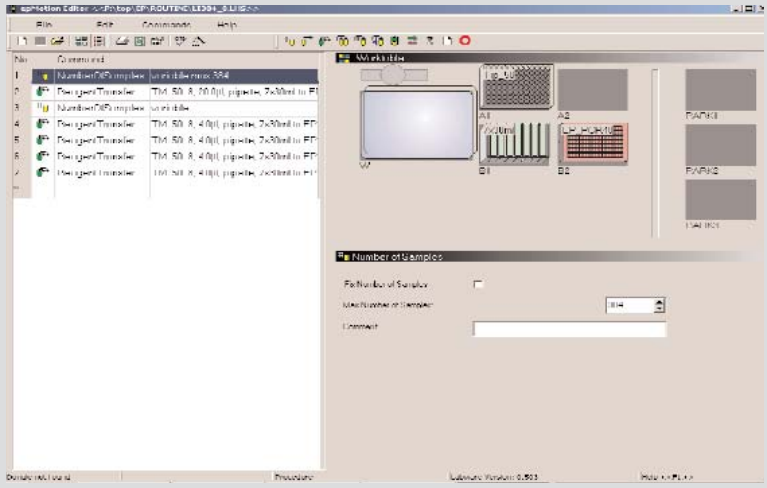

Fig. 1: Worktable set-up (epMotion Editor screen-shot); Pos. A1 50 µl tips; Pos. B1 7x30 ml reservoirs in tub holder; Pos. B2 384-well Eppendorf twin.tec PCR plate; W = waste container

In order to test for the possibility of contamination in the case of glycerol-containing solutions, it is recommended to select the nearly saturated LiCl solution (375 g LiCl/l) as the stock solution. This corresponds to a Li content of 61 mg/ml (equivalent to 61,000,000 ng Li/ml = 61 ng Li/nl). At a density of approx. 1.2 g/ml, the nearly saturated LiCl solution shows similar pipetting behavior as a 40 - 60 % glycerol solution. In contrast, if the test involves the dosing of a rather aqueous medium, the stock solution mentioned above is to be diluted 1 + 9-fold and used in the form of the 1:10 dilution. This corresponds to 6,100,000 ng Li/ml (= 6.1 ng Li/nl). Demineralized water is used as the diluent.

To test for contamination using the epMotion 5070 or 5075, a 384-well PCR plate and dosing tool TS 50-1 or TM 50-8 and the corresponding ep T.I.P.S. 50 µl are used. Either of the two methods, Li384_1 and Li384_8, is employed in the procedure. Both methods are stored in the folder of the ep-node, "Routine", in the form of pre-programmed methods.
Each well then receives 30 µl of demineralized water and subsequently, in a chess board-like pattern, every other well receives 4 µl of water or 4 µl of lithium solution. Since the test well received 30 µl of demineralized water and the measurement at the ELEX requires a liquid volume of at least 1 ml, the contamination test drop has a measured volume of 1030 µl. If a drop with a volume of 0.1 nl (= 6.1 ng Li) of the stock solution described above enters the well containing 30 µl of water (and subsequently is present in 1030 µl volume in the measurement), the flame photometry measurement would generate a stable signal corresponding to approx. 6 ng Li/ml. Using the diluted stock solution (1 + 9), contamination of the well with approx. 0.2 nl (= 1.2 ng Li) would generate a signal just above the detection limit.

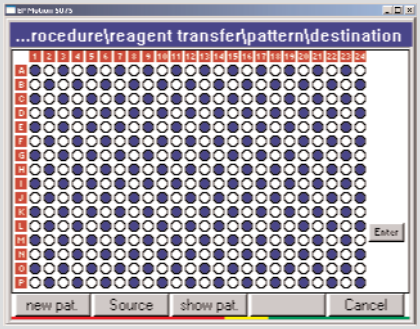

Fig. 2: 384-well Eppendorf twin.tec PCR plate filled in a chess board-like pattern

ELEX 6361 measuring parameters:
For the Li assay, the zero point of the Eppendorf Flame Photometer ELEX was determined using demineralized water. Solutions containing 100, 250, and 500 ng Li/ml were used as the standards. The solution containing 500 ng Li/ml was used for signal amplification (Standard High). Acetylene was used as the combustible gas. The standard Li filter 671 nm was used. Solutions containing 0, 2, 5, and 10 ng Li/ml were used as controls. The Eppendorf Flame Photometer ELEX 6361 was employed in the test. Alternatively, the Li assay can be performed using a similarly sensitive AAS or ICP apparatus.
Flame photometers for Li assays in clinical chemistry for the so-called "Lithium drug monitoring" are not suitable for this contamination test.

Results

In total two out of 384 wells produced signals of up to 2 ng Li/ml (2 ppb). This corresponds to a contamination level of approx. 30 pl for the corresponding well and represents 0.52 % of all wells.

The satellite drops of this size do not produce interference at a level that can be detected in a PCR reaction. This is evident since a PCR reaction was tested in parallel using the same dosing parameters. The gel electrophoretic analysis showed no contamination signals, (Fig. 3).

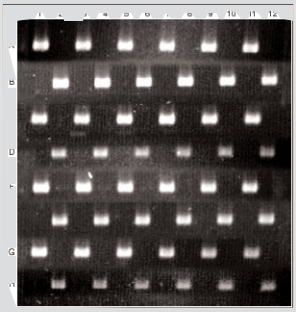

Fig. 3: Amplification of the human ß-globin gene across one fourth of a 384-well PCR plate [2].

We conclude therefore the described method as more sensitive than PCR and providing the evidence for virtually contamination-free pipetting using the epMotion 5070/75 stations.

Literature

[1] H. Specker and H. Kaiser "Zeitschrift für Analytische Chemie" Vol. 149; 1956
[2] Frank Apostel, Eppendorf BioNews 20

For ordering information for the epMotion and accessories, please refer to www.epmotion.com.