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APPLICATION NOTE No. 350

Multipette®/Combitips® System Allows for Fast, Precise and Sterile Liquid Transfer in Cell Culture

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Abstract

Pipetting in cell culture needs the consideration of various factors - mainly sterility, accuracy and precision. Liquid handling systems used in cell culture should assure sterile liquid transfer and accurate and precise pipetting results. Commonly, air-cushion pipettes with filter tips and pipette controllers with serological pipettes are used for liquid transfer in cell culture. This application note introduces a third pipetting system suitable for cell culture applications: Positive displacement dispensers offer the great advantage of complete aerosol prevention. The piston is integrated in the tip of the positive displacement system and hermetically seals the sample in the tip from the instrument. This prevents contamination of the instrument or a cross-contamination between different samples.

In terms of accuracy and precision, positive-displacement dispensers show equivalent results to those obtained with mechanical or electronic air-cushion pipettes and are obviously suitable for handling cells. In contrary, reproducibility of results will be affected when pipette controllers with serological pipettes are used. That highlights that pipette controllers and serological pipettes have clearly to be kept for applications which do not require accuracy and precision e.g. transferring larger volumes of e.g. media out of flasks, dishes or roller bottles.

Introduction

Mammalian cell culture has become a common laboratory technique and is used for a large range of applications such as vaccines production, cell therapy or cancer research. Furthermore, cell cultures offer excellent model systems for studying basic cell physiology (as aging or metabolic activities) and thus play a crucial role in drug discovery processes [1]. As most applications in cell culture include several pipetting steps, choosing the appropriate liquid handling instrument which fulfils the special needs of the application is essential. There are different factors which need to be considered when talking about pipetting in cell culture.

Firstly, as microbiological contamination is the most important concern of the researcher, this is sterility/safety. Sterility is a must in cell culture and all steps in cell culture have to be performed under sterile conditions. Secondly, this is accuracy and precision. Especially when working with small volumes (< 1 mL), a precise and accurate liquid transfer is essential. Last but not least it is time and costs. Depending on the experiment, time is a crucial factor which contributes strongly to the success of an experiment. The time needed for an experiment and its success are furthermore strongly linked to experimental costs.

For all steps in cell culture, the researcher has to decide which of the four mentioned factors is most crucial for the application (mostly one can optimize for two but not for all). Depending on the decision made, the most appropriate liquid handling system should be chosen. Commonly pipette controllers with serological pipettes and classical air-cushion pipettes are used for cell biology applications. The disposable plastic serological pipettes are mainly used for the transfer of larger volumes (~ 1 mL to 50 mL) out of flasks, dishes or roller bottles. Here the priority is speed and sterility. Air-cushion pipettes are mainly used for the transfer of smaller volumes (< 1 mL), where the priority mainly lays on precision and speed. As the greatest sources of microbial contamination are aerosols generated during culture manipulation, the use of filter tips is highly recommended to avoid pipette, and subsequently, sample contamination. Nevertheless, some studies demonstrated that a 100 % protection against contamination cannot be guaranteed by conventional single-layer filter tips. Indeed, it has been shown that for particles of a diameter between 0.3 µm and 0.7 µm (corresponding to the size of many viruses and bacteria), the filter efficiency can drop to 85 % [2].

Consequently researchers should carefully select the consumable used with air-cushion pipettes and if possible should prefer filter tips with a two-phase filter, such as ep Dualfilter T.I.P.S.® by Eppendorf. Here a 99.9 % protection against aerosols is assured.

An alternative to pipette controllers and air-cushion pipettes are positive displacement dispensers. In terms of sterility, these devices offer a significant benefit as a contamination of the instrument or a cross-contamination between different samples via aerosols is prevented. This is ensured by a piston which is integrated in the tip of the positive displacement system and hermetically seals the sample in the tip from the instrument. Furthermore this system allows accurate and precise pipetting results especially when working with liquids whose physical properties differ from those of water. Here, the Eppendorf Multipette® M4 multi-dispenser used with Combitips® advanced dispenser tips in Biopur® quality has been compared to traditionally used pipetting systems in cell culture. In the following, the huge advantages offered by the Multipette/Combitips system in terms of accuracy and precision needed for many cell culture applications are demonstrated.

Materials and Methods

Materials

Pipetting systems (instruments and consumables)

> Eppendorf, Multipette® M4 multi-dispenser
> Eppendorf, Combitips® advanced 2.5 mL Biopur
> Eppendorf, Combitips® advanced 1 mL Biopur
> Eppendorf Research® plus pipette, 20-200 µL
> Eppendorf Xplorer® electronic pipette, 50-1000 µL
> Eppendorf, ep Dualfilter T.I.P.S.®, 2-100 µL PCR clean/sterile
> Eppendorf, ep Dualfilter T.I.P.S.®, 50-1000 µL PCR clean/sterile
> Eppendorf, Easypet® 3 electronic pipette controller
> Eppendorf Serological Pipets, 1 mL

Instrument Calibration

> Mettler-Toledo®, micro balance Excellence plus XP26PC
> VWR, Water Molecular Biology Grade
> MEM medium supplemented with 10% FBS superior

Cell Culture

> Human Embryonic Kidney 293 cells (HEK 293) (DSMZ ACC 305), cultivated in MEM medium supplemented with 10% FBS superior
> Eppendorf, Cell Culture Flask T-75, TC treated, with filter cap (discontinued in 2021)

Cell Counting
> Roche® Innovatis, CASY® Cell Counter and Analyser, model TT 150 µm
> Roche Innovatis, CASY ton
> Roche Innovatis, CASY cups

WST-1 Colorimetric Assay
> Roche Diagnostics, Cell Proliferation Reagent WST-1
> Eppendorf, Cell Culture Plate, 24-Well, TC treated
> Eppendorf, PlateReader AF2200 (discontinued in 2015)

Methods

Instrument Calibration

Systematic and random errors were determined by gravimetric method in accordance with the EN ISO 8655:2002 standard [3]. As requested by the norm, tests were carried out in a draught-free room. During testing, relative humidity was above 50 % and temperature was constant. Instruments, consumables and test liquids were equilibrated to the test room for at least 2 hours before starting the test. To determine errors, the test liquid was dispensed ten times into a vessel and weighed. For each condition, three series of ten dispensings were performed. A new consumable was used for each series. The systematic error (inaccuracy) and the random error (imprecision) were determined for each series of 10 measurements. Three values were obtained per condition, from which the average and standard deviation were calculated.

Cell Counting

Cell numbers were determined using the CASY Cell Counter and Analyser. Measurement was performed by suspending 100 µL cell suspension in 10 mL of CASY ton, an electrolyte developed specifically for cell counting and by aspirating them through a measuring pore. During the measurement process, a pulsed low voltage field is applied to the measuring pore via two platinum electrodes. The measuring pore filled with electrolyte solution represents a defined electrical resistance. During their passage through the measuring pore, cells displace a quantity of electrolyte corresponding to their volume. Since intact cells are reacting as isolators, an increased level of resistance is achieved over the measuring pore.

Cell seeding and WST-1 Colorimetric Assay

After cell counting, a cell suspension of $1.5x10^6$ cells/mL was prepared for cell seeding. From this stock solution, various defined cell numbers were seeded into 24-well plates. In order to evaluate the number of viable cells, 40 µL of WST-1 (Water Soluble Tetrazolium salt) was added per well. As shown on figure 1, the WST-1 is cleaved to formazan by cellular enzymes. This reduction is largely dependent on the NAD(P)H production in viable cells. Therefore, the amount of formazan dye formed directly correlates to the number of metabolically active cells in the culture.

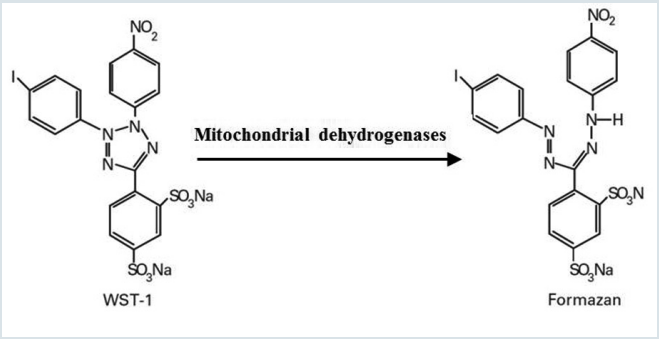

Figure 1: Cleavage of the tetrazolium salt WST-1 to formazan

Cell culture plates were incubated with the WST-1 reagent for 3 hours in a humidified atmosphere (37 °C, 5 % CO_2). Afterwards, plates were mixed for 1 minute in the PlateReader AF2200 and read at 450 nm with a reference reading at 690 nm. The measured absorbance can be directly correlated to the number of viable cells.

Results and Discussion

Performance comparison of different pipetting systems
Accuracy and precision of the instruments compared within this study were established by usual gravimetric method according to the EN ISO 8655:2002 norm. The volume tested was 100 µL. Each instrument was used with its consumable: Multipette M4 with Combitips advanced 1 mL, Research plus single-channel pipette with 100 µL tips, Xplorer single-channel pipette with 1000 µL tips and Easypet® 3 with 1 mL serological pipettes. For each condition, three series of ten dispensings were performed.

As expected, the serological pipette controlled by the Easypet 3 delivers the least reproducible results of all four systems tested (Figure 2). The volume delivered with this system is considerably higher and a constant and stable dispensing is difficult to acquire. Depending on the application, this performance could have an impact on the result. On the opposite, with mean systematic errors below 0.6 %, air-cushion pipettes and the Multipette/Combitips system appear as the more reliable systems. This data set demonstrates that a more accurate and precise liquid transfer can be guaranteed with a positive-displacement instrument as well as with an air-cushion system.

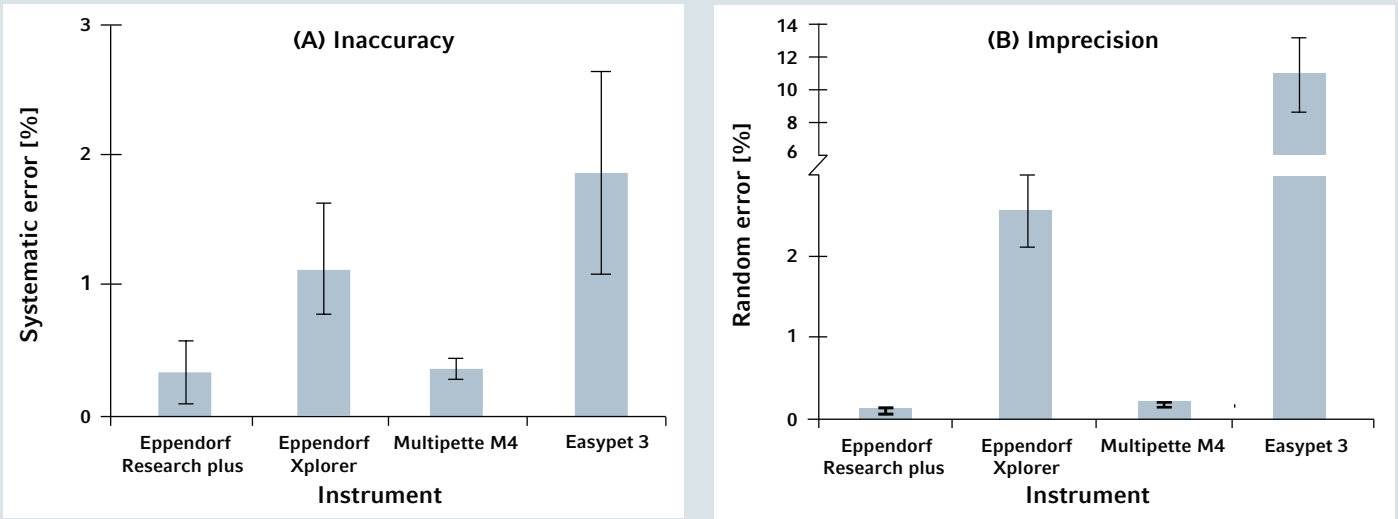

Figure 2: Inaccuracy (A) and imprecision (B) at 100 µL measured for the different pipetting system used (n=3).

Impact of the pipetting system on cell counting
To evaluate the impact of the pipetting system on cell counting, three systems have been compared: a mechanical air-cushion pipette (Eppendorf Research® plus) with ep Dualfilter T.I.P.S. filter pipette tips, a positive displacement system (Multipette M4) with Combitips advanced and an electronic pipette controller (Easypet 3) used with serological pipettes. The number of HEK 293 cells was determined by using the CASY technology.

Table 1: Cell counting results obtained with different pipetting systems (n=30).

Pipetting system	Mean Cell Number	CV	Min value	Max value	Mean Viability
Air-cushion	1.65x10⁶ cells/mL	3.0 %	1.52 10⁶ cells/mL	1.75 10⁶ cells/mL	96.4 %
Positive displacement	1.62x10⁶ cells/mL	2.5 %	1.53 10⁶ cells/mL	1.69 10⁶ cells/mL	96.0 %
Pipette Controller	1.50x10⁶ cells/mL	6.8 %	1.39 10⁶ cells/mL	1.85 10⁶ cells/mL	96.2 %

While the cell viability is not affected, the cell number is influenced by the pipetting system used to dispense cell suspension for cell counting. The data indicates that the most precise system is the Multipette/Combitips system while a pipette controller in combination with serological pipette delivers the least reproducible results. As Research plus pipettes are classically used for cell counting, they are considered as reference instruments. A Fisher test for statistical analysis revealed that the positive displacement dispenser and air-cushion pipette induce an equivalent variability of cell counting results, while the reproducibility obtained with the pipette controller is significantly lower (data not shown). These results correlate with the previous calibration data: air-cushion pipettes and positive displacement dispensers are the liquid handling tools of choice for accurate and precise liquid transfer for cell culture applications.

Impact of the pipetting system on cell seeding

Based on the cell counting data, six cell suspensions of 1.5x10⁶ cells/mL were prepared by considering the minimal and the maximal counting values obtained for each pipetting system (table 1). With those cell suspensions various cell amounts were seeded in 24-well plates (Table 2) using the following pipetting systems: an electronic air-cushion pipette Eppendorf Xplorer® with ep Dualfilter T.I.P.S. filter pipette tips, a positive displacement system (Multipette M4) with Combitips advanced dispenser tips and an electronic pipette controller (Easypet 3) with serological pipettes.

Table 2: Number of cells per well and corresponding cell and media volume

Number of cells/well	Cell volume dispensed	Media volume dispensed
0	0 µL	400 µL
75,000	50 µL	350 µL
150,000	100 µL	300 µL
300,000	200 µL	200 µL
450,000	300 µL	100 µL
600,000	400 µL	0 µL

The cell proliferation of seeded cells was analysed with a WST-1 Colorimetric Assay (Figure 3).

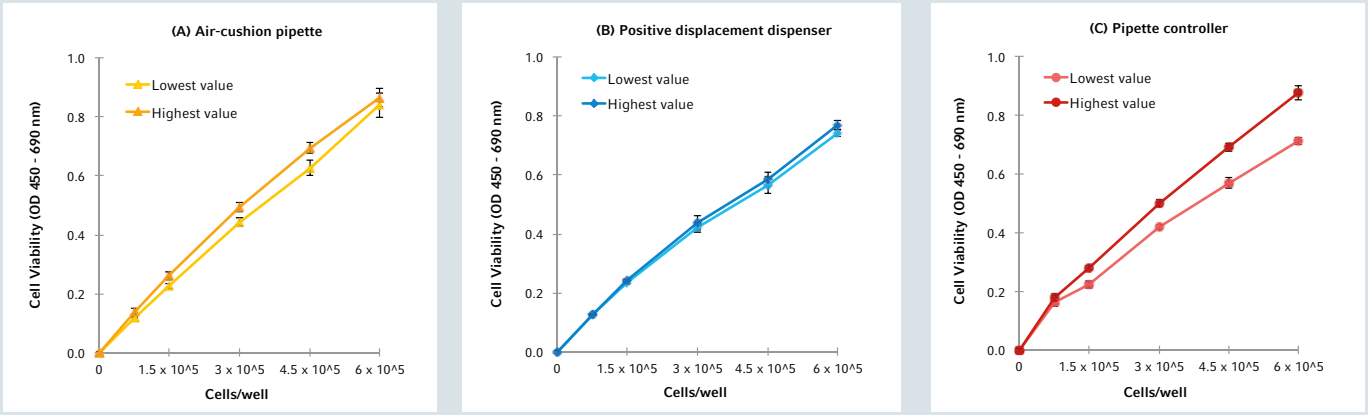

Figure 3: Cell proliferation results of WST-1 colorimetric assay for increasing seeded cell numbers using (A) air-cushion pipette, (B) positive displacement dispenser and (C) pipette controller (n=4).

The measured absorbance correlates with the number of viable cells after seeding with different pipetting systems (Figure 3). These results indicate that using a positive-displacement system, for cell counting as well as for cell seeding, ensures reproducibility of the final result. Curves obtained with the multi-dispenser used are similar or even slightly less variable than those obtained with the air-cushion pipette. On the opposite, as soon as a less precise instrument as pipette controller is used, reproducibility of the final results will be affected. That highlights that serological pipettes have clearly to be kept for liquid transfer steps which do not require accuracy and precision – e.g. transferring larger volumes of the media out of flasks, dishes or roller bottles. On the contrary, the Multipette/Combitips system combines accuracy, precision, and contamination prevention and represents a perfect alternative for routine tasks requiring a liquid handling device in cellular biology labs. Electronic variants like the Multipette E3x allow additionally to set low, controlled and reproducible piston speeds, which can be hardly achieved with mechanical systems. A slower speed offer flow velocites that are slow enough to minimize shear stress on cells [4].

Conclusion

Liquid transfer in cell culture has to fulfill different requirements, among which the most important are sterility/safety, accuracy and precision. In this study different pipetting systems were tested for routine applications in cell culture: air-cushion pipettes, which are commonly used to pipette smaller volumes, pipette controllers commonly used for larger volumes like media and positive displacement dispensers. The data set shows that serological pipettes handled by a pipette controller should be limited to applications which do not demand a high level of reproducibility. We furthermore demonstrated that the Multipette/Combitips system is ideal for handling cells. The performance of this pipetting system in cell culture is equivalent to that of air-cushion pipettes. As Combitips advanced prevent any contact between the sample and the device, the Multipette M4 appears as the perfect alternative for scientists particularly concerned by contamination.

Literature

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