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Multipette®/ Repeater® M4 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. The most important ones are 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 in combination with filter tips and pipet controllers in combination with serological pipets are used for liquid handling transfer in cell culture. This application note introduces a third pipetting system suitable for cell culture applications. These are positive displacement dispensers which offer the great advantage of complete aerosol prevention as the piston integrated in the tip of the positive displacement system hermetically seals the sample in the tip from the instrument and thus ensures that no contamination of the instrument or a cross-contamination between different samples will occur.

In terms of accuracy and precision positive-displacement dispenser show equivalent results to those obtained with manual or electronic air-cushion pipettes and are obviously suitable for handling cells.
In contrary, reproducibility of results will be affected when pipet controllers in combination with serological pipets are used. That highlights that pipet controllers and serological pipets 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

Animal 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 his application (mostly you can optimize for two but not for all). Depending on the decision made, the most appropriate liquid handling device should be chosen. Commonly pipet controllers in combination with serological pipets and classical air-cushion pipettes are used for cell biology applications. The disposable plastic serological pipets in combination with pipette controllers 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 Eppendorf ep Dualfilter T.I.P.S.®. Here a 99.9 % protection against aerosols is assured.

An alternative to pipet 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 completely 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. In this Application note, the Eppendorf Multipette/ Repeater M4 Dispenser used in combination with Combitips advanced® Biopur® has been compared to traditionally used pipetting systems in cell culture. This is to demonstrate the huge advantages offered by Multipette M4 in terms of accuracy and precision needed for many cell culture applications.

Materials and Methods

Materials

Pipetting systems (instruments and consumables)

> Eppendorf, Multipette M4 (order no. 4982 000.012)
> Eppendorf, Combitips advanced 2.5 mL Biopur (order no. 0030 089.650)
> Eppendorf, Combitips advanced 1 mL Biopur (order no. 0030 089.642)
> Eppendorf, Research® plus pipette, 20-200 µL (order no. 3120 000.054)
> Eppendorf, Xplorer® pipette, 50-1000 µL (order no. 4861 000.040)
> Eppendorf, ep Dualfilter T.I.P.S., 2-100 µL PCR clean/sterile (order no. 0030 077.547)
> Eppendorf, ep Dualfilter T.I.P.S., 50-1000 µL PCR clean/ sterile (order no. 0030 077.571)
> Eppendorf, Easypet® 3 (order no. 4430 000.018)
> Eppendorf, Serological Pipets, 1 mL (order no. 0030 127.692)

Instrument Calibration

> Mettler-Toledo®, micro balance Excellence plus XP26PC (Mettler-Toledo, order no. 11106021)
> VWR, Water Molecular Biology Grade (order no. 733-1090)
> 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 (order no. 0030 711.122)

Cell Counting
> Roche® Innovatis, CASY® Cell Counter and Analyser, model TT 150 µm (order no. 4 00 06)
> Roche Innovatis, CASY ton (order no. 4 30 01)
> Roche Innovatis, CASY cups (order no. 4 30 03)

WST-1 Colorimetric Assay
> Roche Diagnostics, Cell Proliferation Reagent WST-1 (order no. 11 644 807 001)
> Eppendorf, Cell Culture Plate, 24-Well, TC treated (order no. 0030 722.116)
> Eppendorf, PlateReader AF2200 (order no. 6141 000.002)

Methods

Instrument Calibration

Systematic and random errors were determined by gravimetric method in accordance with the EN ISO 8655 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 number was determined by using the CASY Cell Counter and Analyser. Measurement was performed by suspending 100 µL of 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 quantities of cells 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.

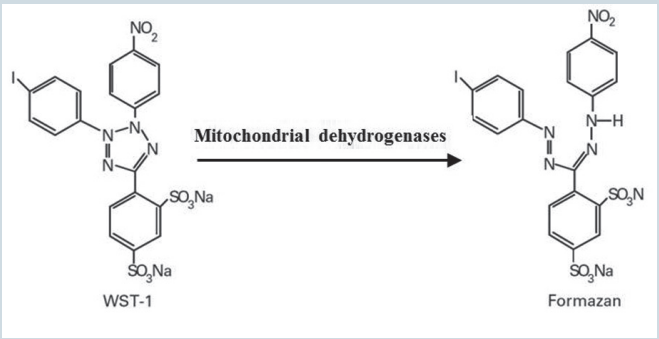


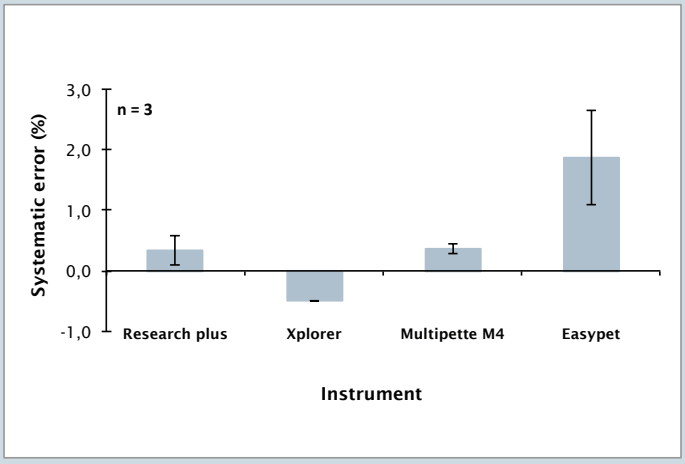
Figure 1: Cleavage of the tetrazolium salt WST-1 to formazan

Cell culture plates were incubated with the WST-1 reagent for 3 hours in a humidified atmosphere (37 °C, 5 % CO_2). After finalizing the incubation time, the plates were mixed for 1 minute in the PlateReader AF2200 and afterwards 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 evaluation of the used pipetting systems
Accuracy and precision of the instruments compared within this study were established by usual gravimetric method in accordance with the EN ISO 8655 norm. The volume tested was 100 µL. For this purpose, each instrument was associated with its dedicated consumable (Multipette/Repeater 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 serological pipets of 1 mL). For each condition, three series of ten dispensings were performed.

As expected, the serological pipet controlled by the Easypet 3 is the less accurate and the less reproducible system of all four systems tested. The volume delivered with this system is systematically higher and a constant and stable dispensing is difficult to acquire. Depending on the application, those poor performances could have an impact on the application result. On the opposite, with mean accuracy and precision values below 0.6 %, air-cushion pipettes and Multipette/Repeater M4 appear as the most reliable systems. Those data obviously demonstrate that an accurate and precise liquid transfer can be guaranteed with a positive-displacement instrument as well as with a traditional air-cushion device.



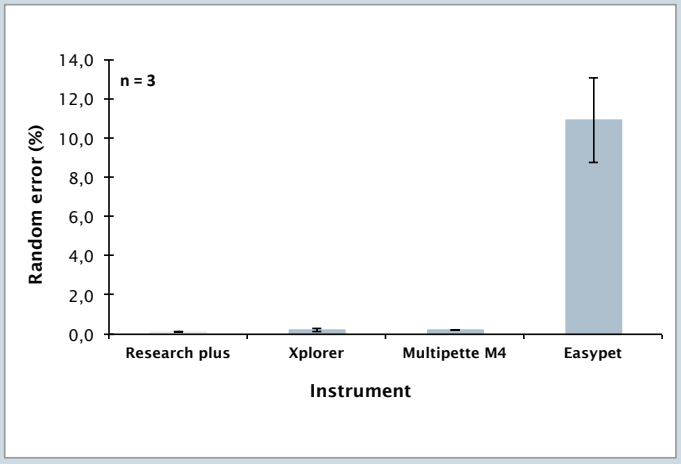


Figure 2 and 3: Inaccuracy (Figure 2) and imprecision (Figure 3) at 100 µL measured for the different pipetting system used

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 manual air-cushion pipette (Research plus pipette) with ep Dualfilter T.I.P.S., a positive-displacement system (Multipette/Repeater M4) combined with Combitips advanced and an electronic pipet controller (Easypet 3) associated with serological pipets. The cell number of the HEK 293 cells was determined by using the CASY technology. Measurements were performed by dispensing 100 µL of the cell suspension into 10 ml of CASY ton and aspirating this solution through the measuring pore of the CASY cell counter.

Table 1: Cell counting results obtained according to pipetting system used to dispense 100 µL of the cell solution (n=30).

Pipetting system n=30	Mean Cell Number	CV	Min value	Max value	Mean Viability
Air-displacement	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 %

As shown in table 1, the level of cell viability is not affected by the pipetting system used as the percentage of viable cells is around 96 % for all conditions assessed. In contrast, the cell count variability is obviously influenced by the instrument used to dispense the cell suspension. Indeed, data indicate that the most precise system is the Multipette/Repeater M4 associated with Combitips advanced while a pipet controller in combination with serological pipets is the least reproducible system. As Research plus pipettes are classically used for cell counting, they are considered as reference instruments. A Fisher test has been applied to define if reproducibility offered by all other systems is significantly different from this reference (data not shown). According to this statistical test, positive-displacement dispenser and air-cushion pipettes induce an equivalent variability of cell counting results while the reproducibility obtained with the pipet controller is significantly lower. Those results can be correlated with previous calibration data and prove that the performance of a pipetting system has an impact on the application. Depending on the pipetting system used the impact is of different magnitude.

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). Those cell suspensions were used to seed various cell amounts into 24-well plates. Table 2 details the numbers of cells seeded and the corresponding cell suspension volumes. Three pipetting systems were used to dispense increasing numbers of cells: an electronic air-cushion pipette (Xplorer single-channel pipette) used with ep Dualfilter T.I.P.S., a positive-displacement system (Multipette/ Repeater M4) combined with Combitips advanced and an electronic pipet controller (Easypet 3) associated with serological pipets. In every well, the final culture volume has been adjusted to 400 µL with culture media as described in table 2. Four replicates were performed for each condition.

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

In order to evaluate the number of viable cells in every well, 40 µL/well of WST-1 (Water Soluble Tetrazolium salt) was added. As shown in figure 1, the WST-1 is cleaved to formazan dye by cellular enzymes. The absorbance of this dye is measured and can directly be correlated to the number of viable cells. Curves obtained are depicted in figures 4 to 6.

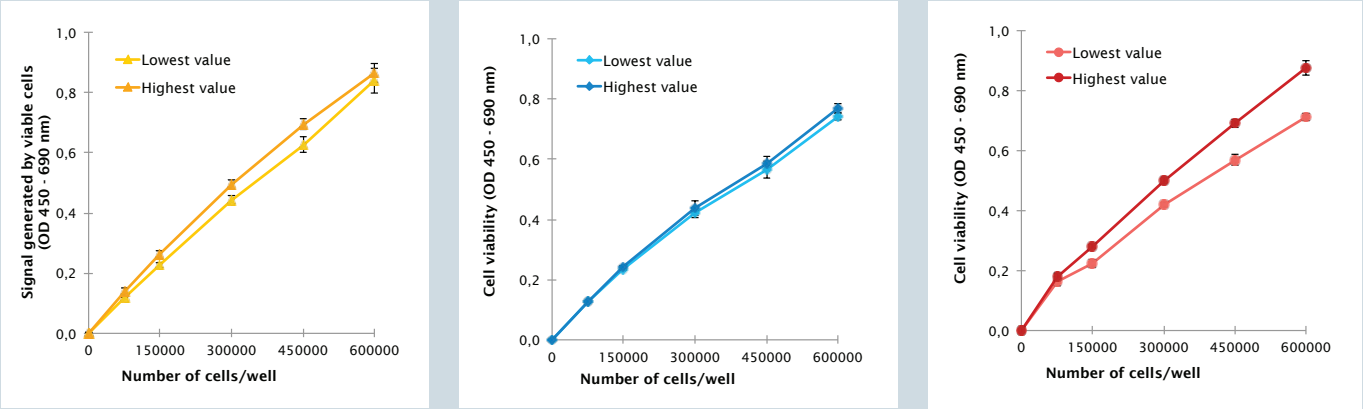


Figure 4 - 6: Signal curves generated by increasing numbers of cells. Three different pipetting systems were used for cell counting as well as cell seeding (air-cushion system, figure 4), (positive-displacement system, figure 5) and (pipet controller, figure 6).

These results indicate that using a positive-displacement system, as the Multipette/Repeater M4 in combination with Combitips advanced, for cell counting as well as for cell seeding, guarantees reproducibility of the final result. Curves obtained with the dispenser are similar or even slightly less variable than those obtained with air-cushion instruments. On the opposite, as soon as a less precise instrument as a pipet controller is used, reproducibility of the final results will be affected. That highlights that serological pipets have clearly to be kept for activities which do not require accuracy and precision e.g. transferring larger volumes of e.g. media out of flasks, dishes or roller bottles. On the contrary, because it combines accuracy, precision and contamination prevention, the Multipette/Repeater M4 in combination with Combitips advanced represents a perfect alternative for all routine tasks requiring a liquid handling device in cellular biology labs.

Conclusion

Liquid handling transfer in cell culture has to fulfill different requirements, among which the most important are sterility/safety, accuracy and precision. In this study we tested three different pipetting systems, air-cushion pipettes which are commonly used to pipette smaller volumes, pipet controller commonly used for larger volumes of e.g. media and positive-displacement dispenser. We were able to show that serological pipets handled by a pipet controller should be limited to applications which do not demand a high level of reproducibility. We furthermore demonstrated that the Multipette/Repeater M4 combined with Combitips advanced is obviously suitable for handling cells. Performances offered by this pipetting system are equivalent to those obtained with manual or electronic air-cushion pipettes. Moreover, because the Combitips advanced prevent any contact between the sample and the device, the Multipette/Repeater M4 appears as the perfect alternative for scientists particularly concerned by contamination.

Literature

[1] Nema R, Khare S. An animal cell culture: Advance technology for modern research. Advances in Bioscience and Biotechnology 2012; 3:219-226.
[2] Le Rouzic E, Contamination-pipetting: relative efficiency of filter tips compared to Microman® positive displacement pipette. Nature methods 2006; 3 Application note.
[3] EN ISO 8655, Parts 1-6: Piston-operated volumetric apparatus. © ISO 2002.

Ordering information

Description		Order no. international	Order no. North America
Multipette® M4 incl. holder (for wall and/or pipette carousel)		4982 000.012	
Repeater® M4 Starter Kit, Repeater® M4, Combitip Rack, Combitip assortment pack			4982000322
Multipette® **Repeater® E3**		4987 000.010	4987000118
Research® plus pipette 20-200 µL		3120 000.054	3120000054
Xplorer® pipette 50-1000 µL		4861 000.040	4861000732
ep Dualfilter T.I.P.S.® 2-100 µL PCR clean/sterile		0030 077.547	022491296
ep Dualfilter T.I.P.S.® 50-1000 µL PCR clean/sterile		0030 077.571	022491253
Easypet® 3		4430 000.018	4430000026
Combitips advanced® in Eppendorf quality			
	0.1 mL	0030 089.405	5392070020
	0.2 mL	0030 089.413	5392070011
	0.5 mL	0030 089.421	0030089421
	1mL	0030 089.430	0030089430
	2.5 mL	0030 089.448	0030089448
	5 mL	0030 089.456	0030089456
	10 mL	0030 089.464	0030089464
	25 mL	0030 089.472	0030089472
	50 mL	0030 089.480	0030089480
Combitips advanced® PCR clean			
	0.1 mL	0030 089.766	0030 089766
	0.2 mL	0030 089.774	0030 089774
	0.5 mL	0030 089.782	0030 089782
	1 mL	0030 089.790	0030 089790
	2.5 mL	0030 089.804	0030 089804
	5 mL	0030 089.812	0030 089812
	10 mL	0030 089.820	0030 089820
	25 mL	0030 089.839	0030 089839
	50 mL	0030 089.847	0030 089847
Combitips advanced® Biopur			
	0.1 mL	0030 089.618	0030 089618
	0.2 mL	0030 089.626	0030 089626
	0.5 mL	0030 089.634	0030 089634
	1 mL	0030 089.642	0030 089642
	2.5 mL	0030 089.650	0030 089650
	5 mL	0030 089.669	0030 089669
	10 mL	0030 089.677	0030 089677
	25 mL	0030 089.685	0030 089685
	50 mL	0030 089.693	0030 089693
25 mL adapter (1 pc.)		0030 089.715	0030 089715
50 mL adapter (1 pc.)		0030 089.723	0030 089723
25 mL adapter (7 pc.), Biopur		0030 089.731	0030 089731
50 mL adapter (7 pc.), Biopur		0030 089.740	0030 089740
Combitip Rack		0030 089.758	0030 089758
Serological Pipets, 1 mL		0030 127.692	0030127692
Serological Pipets, 2 mL		0030 127.706	0030 127706
Serological Pipets, 5 mL		0030 127.714	0030 127714
Serological Pipets, 10 mL		0030 127.722	0030 127722
Serological Pipets, 25 mL		0030 127.730	0030 127730
Serological Pipets, 50 mL		0030 127.749	0030 127749

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