Applications

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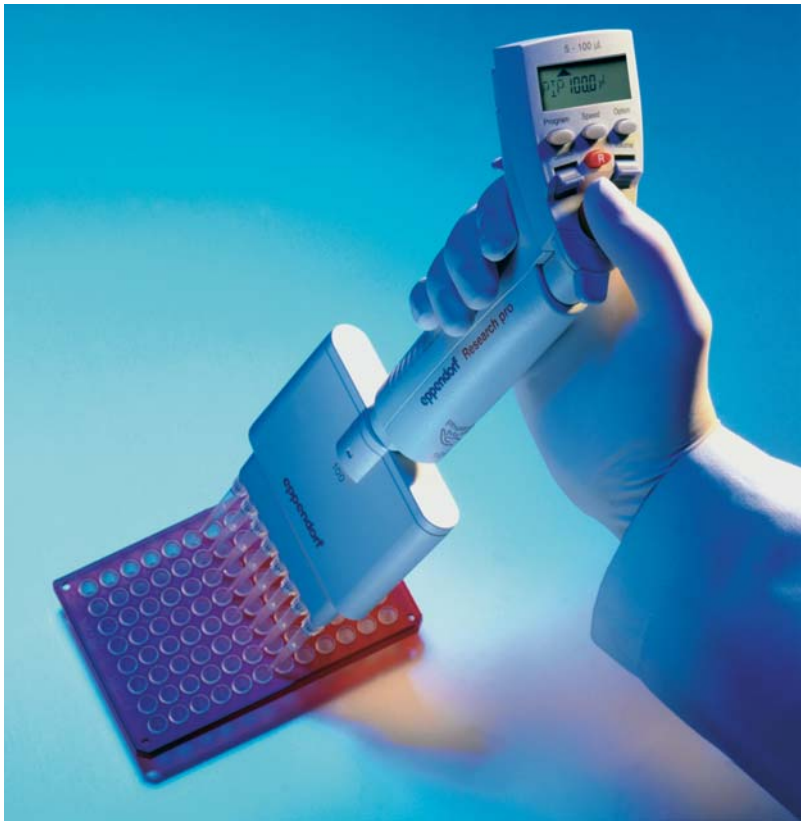
Comparison of Pipetting Characteristics of the Electronic Pipette Eppendorf Research® pro and the Manual Pipette Eppendorf Research® using an Enzyme-Linked ImmunoSorbent Assay (ELISA)

Gmeinder A., Kania M., Rentschler Biotechnologie GmbH, Laupheim, Germany;
Kornelia Ewald, Eppendorf AG, Hamburg, Germany

Introduction

Depending on the individual applications in the pharmaceutical industry pipetting is an essential component of everyday work.
One of the main requirements on pipettes is the correct pipetting of solutions in quantitative terms. During everyday work at the laboratory the pipetting of a whole range of different solutions and buffers is carried out under Good Manufacturing / Laboratory Practise conditions. They can be roughly divided up into aqueous, protein-containing and detergent-containing solutions depending on the main constituent although there are no firm boundaries between them here.

Besides the factors of accuracy and precision the time requirement and the aspect of ergonomics also play a major role for routine pipetting.
This study looks at the electronic pipette Eppendorf Research® pro (single and multi-channel) and the manual pipette Eppendorf Research® (single and multi-channel) as regards these parameters and makes a comparison between the accuracy and time-specific data of the two pipettes.
Eppendorf PhysioCare Concept pipettes were used for all preparations. The Eppendorf PhysioCare Concept (PCC) is the new standard for integrated liquid handling systems and work processes. One of the key feature of this standard is the minimal user exertion. With manual pipettes this means 50 % less effort has to be applied when aspirating and dispensing liquids as well as a considerable reduction in the force used for attaching and ejecting tips with all PCC pipettes.



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Material and Methods

Material
Pipettes:
Manual pipettes
- Eppendorf Research® fixed-volume single-channel 50 µl
- Eppendorf Research® adjustable 8-channel 30 – 300 µl

Electronic pipettes
- Eppendorf Research® pro single-channel 20 – 300 µl
- Eppendorf Research® pro 8-channel 20 – 300 µl
- Eppendorf Research® pro 8-channel 50 – 1,200 µl

Buffer:
Aqueous: 70 mM phosphate buffered saline (PBS)
Protein-containing: 1 % bovine serum albumin (BSA) in PBS
Detergent-containing: 0,05 % Tween 20 in PBS

Methods
Examination of accuracy:
The systematic measurement deviation (inaccuracy) of the pipettes was determined gravimetrically. For this purpose the volumes 300 µl and 50 µl of the different solutions were pipetted twenty times into a supply vessel. Beforehand the weight of the vessel was determined, and the Sartorius balance calibrated accordingly. The preparation was repeated seven times, and the weight recorded. The density of each buffer was determined and the nominal value in grams calculated from this. This was followed by calculation of the percentage-based deviation of the actual value from the nominal value. Here the random measurement deviation (imprecision) of the pipettes used was also checked. This is a measure of the percentage-based deviation of the measured values to each other. The experiments were performed using the manual Eppendorf Research® adjustable multi-channel 30 – 300 µl and the electronic pipette Eppendorf Research® pro multi-channel 20 – 300 µl.

Examination of ELISA time comparison:
Here two Enzyme-Linked Immuno-Sorbent Assays (ELISA) were each carried out in parallel using a 96-well ELISA plate. The ELISA process consisted of a total of seven steps, i.e. coating, saturation, sample application, immune reaction, detection, substrate reaction and stopping. The time taken for each pipetting step was documented.

2 ELISA test plates underwent pipetting in parallel: one plate with the manual pipette Eppendorf Research® adjustable and one plate with the electronic pipette Eppendorf Research® pro.
Sample dispensing in the individual wells was performed using the Research® fixed-volume pipette 50 µl and with the Research® pro single-channel pipette 20 – 300 µl.
In all other pipetting steps the twelve 8-well rows in the Elisa plate were filled with identical volumes, on the one hand using the electronic, and on the other, the manual Research® multi-channel pipette. The application times for the different pipettes were determined using a stopwatch and then compared with each other.

Results and Discussion

As can be seen in table 1, the electronic pipette Research® pro complies with the specified limits despite different buffer groups and different volumes (50 µl and 300 µl) and continuously provides accurate results. The manual pipette Research® was also within the limits for the volume size 300 µl with all three buffer groups. In the lower volume range of 50 µl the level of inaccuracy was ≤ 3 % for all three buffer groups. Here a key role is played by the different types of buffer, which are in some cases highly viscous (e.g. protein-containing buffer), thus making pipetting very difficult. To obtain better results here reverse pipetting could be carried out. With *reverse pipetting* the liquid is aspirated with blow-out and dispensed without blow-out. This causes the tip to retain liquid, which is then discarded. The disadvantage of the manual pipette is that it is not possible to set a fixed speed in comparison with the electronic pipette. The pipetting speed is controlled by the user and is thus highly dependent on operation and external influences.

Table 1: Systematic and random measurement deviation of Eppendorf Research® pro and the Research® multi-channel pipettes for the different buffers

	Research® pro multi-channel Systematic measurement deviation (inaccuracy)		Research® multi-channel Systematic measurement deviation (inaccuracy)		Research® pro multi-channel Random measurement deviation (imprecision)		Research® multi-channel Random measurement deviation (imprecision)	
Pipetting volume	50 µl	300 µl	50 µl	300 µl	50 µl	300 µl	50 µl	300 µl
Buffer group protein-containing inaccuracy [%]	**2.1**	**2.0**	**7.9**	**1.5**	**0.6**	**0.3**	**0.7**	**0.1**
Buffer group aqueous inaccuracy [%]	**0.9**	**1.8**	**4.6**	**1.5**	**1.0**	**0.1**	**0.6**	**0.2**
Buffer group detergent-containing inaccuracy [%]	**0.7**	**1.5**	**5.9**	**0.8**	**0.7**	**0.1**	**0.6**	**0.2**

With the electronic pipette Research® pro 10 different levels can be selected for both the aspirating and the dispensing speed. This allows the pipetting speed to be adjusted to the respective medium, e.g. the viscosity of the buffer. The motorized drive means that the pipetting speed remains constant, thus resulting in improved precision and accuracy. This minimizes user errors to a significant extent.

In comparison with the manual Research pipette, the Research® pro pipette offers more precise results, as well as key benefits such as a time saving due to single aspiration of liquids and serial dispensing, as well as programmability and the variable automatic speed selection mentioned above.

ELISA Time Comparison

With the ELISA time comparison the Research® pro pipette has significant advantages over the manual multi-channel-pipette. It can offer a major time saving through dispensing in several dispensing steps one after the other. With the manual Research pipette on the other hand, the liquid has to be re-aspirated after every dispensing step. The advantage in terms of time is very evident in routine work when using ELISA plates with large quantities.

Compared with the manual multi-channel pipette, the time saving for pipetting with the Research® pro when performing Elisa is approx. 5 minutes (Tab. 2). This offers major economic benefits with a high sample throughput in routine work.

Table 2: Time requirement for performance of necessary pipetting steps in ELISA when using Eppendorf Research® pro and Eppendorf Research® fixed-volume

ELISA work steps	**Time requirement Research® pro** (single and multi-channel) [s]	**Time requirement Research® fixed-volume** (single and multi-channel) [s]
Coating (50 µl)	19	45
Saturation (200 µl)	28	56
Sample application (50 µl) (96 wells)	180	240
1st antibody (50 µl)	19	46
2nd antibody (50 µl)	20	48
Substrate (100 µl)	21	59
Stop solution (50 µl)	20	50
Total time requirement [s]	**307**	**544**

Outlook

It should be borne in mind that although a pipette is primarily a precision tool, it is also an integral part of a work process performed by human beings.
Unlike mechanical pipettes, electronic pipettes can be used to carry out not only basic pipetting functions but also numerous special functions and programs.
Due to the integrated dispensing mode Research pro results in a time saving that is of major significance in laboratories with a high sample throughput. A high level of precision and accuracy of results is achieved thanks to the constant pipetting speed, which can be selected according to the respective medium. With the use of Eppendorf Physio-Care Concept pipettes it was also possible to take the ergonomic aspect into account for pipetting given the considerable reduction in operating force.