8. Safety in pipetting

Mannonen, S., Ph.D. and Syrjä K., B.Sc. Biohit Plc.

Pipetting with air displacement pipettors is one of the most common laboratory tasks and includes a number of potential risks. The risks can be divided into three defined areas: Physical risk through stress (ergonomics), situations where the user is at risk of contamination through handling of infectious or toxic agents, and situations where the sample or specimen is at risk through contamination from other samples or environmental factors. Often the user is well protected by appropriate working benches, seats, clothing and shields, but the pipettor is forgotten.

Modern air displacement pipettors with disposable tips enable fast and accurate pipetting, but contamination of the pipettors may increase both the possibility of unreliable results and health risks in laboratory work. Scientific literature on the contamination of modern air displacement pipettors hardly exists.

The ergonomics of pipetting

Twenty years of pipetting history has demonstrated that the risk of Repetitive Strain Injuries (RSI) increases among those who pipette a lot. Also scientific studies have shown an increased susceptibility of pipettor users to Work Related Upper Limb Disorders (WRULD)(1,2). These disorders have been attributed to a number of factors involved in pipetting. Firstly, whether the person is predisposed towards this type of ailment or injury, secondly, the type and duration of the work, and thirdly, the tool in use. The weight required to depress the plunger on a mechanical pipettor is between 0.5 kg and 5 kg. If this is repeated several hundred times per day it is quite reasonable to expect serious fatigue in the muscles of the hand, wrist and arm. Over 300 hours pipetting/year increases the risk of strain injuries in the hand and shoulder area. This problem can be caused by a number of elements: Design of the pipettor, number of repetitions, seating position, lack of rest intervals or poor bench layout. It can be also accompanied by other ailments such as backaches or headaches.

Research undertaken at a leading ergonomics institute (1) has provided valuable information concerning the elements of the design of commercial products that may lead to these problems. Features which were identified to make plunger operated pipettors more difficult to use are plunger operation itself, tip ejection, heavy and cumbersome grip design, and volume setting.

The Biohit electronic pipettors overcome most of these problems (Fig. 1). The plunger is operated by a motor which is controlled by a microprocessor, and only a light touch is needed to operate the pipettor. The tip ejection can be done using the whole hand, which eliminates the stress for the thumb unavoidable in mechanical pipettors. The grip is better because of the ergonomic finger support and rather thin handle, which allows the thumb to rest on the start button. As to size there is no increase compared to conventional devices. Even if the pipettor is a bit heavier than the lightest mechanical pipettors on the market, it is much lighter to use than a mechanical pipettor and fits comfortably in the hand. The volume is adjusted simply by pushing the button and the volume starts to run on the display. Moreover, the results are always accurate and reproducible.

Fig. 1. The benefits of an electronic pipettor are in its ergonomics.

Contamination

The user should be fully conversant with the potential hazards contained within the specimen or the sample. Accidental spillage, inhalation or penetration of the skin and containment procedures are dangers that need addressing at a laboratory safety level. The liquid handling element of the procedure should present as few potential hazards as possible to the user. Appropriate working benches, seats, clothing and shields should be used for each type of work to protect the user. On the other hand, good laboratory practice - pipetting slowly and carefully to minimise aerosol formation and foaming - is important to avoid contamination of the pipettor and the subsequent sample.

This may not be sufficient enough and aerosol-barriers, such as filters, between the sample and the pipettor have been introduced. Tip manufacturers have developed aerosol-barrier tips, which are useful in contamination sensitive work, like PCR, RIA and bacteriological work (Fig 2.). The tips have a porous filter positioned inside the tip. During pipetting air flows through the filter, aimed to reduce the flow of aerosols or liquid into the pipettor barrel and subsequently to the next sample (carryover contamination). The material and the features of the filter tips on the markets vary. The small pore size of some filters reduces pipetting speed due to slow air flow through the filter. On the other hand, the pore size in some filters is large enough to let small molecules and aerosols pass through easily. Moreover, using filter tips instead of standard tips is rather expensive.

An alternative to the filtered tips is provided with pipettor that feature a tip cone design that is adapted for the placement of a protective filter (Fig. 3). Two types of filters are available for Biohit pipettors: a standard Safe-Cone filter (PE) and the Biohit Safe-Cone Plus (SCF) filter.

Tip cone filters protect from contamination and carryover

The Biohit standard and Plus filters have a different barrier capacity in a situation simulating overdraw of liquid. The standard filter allows the liquid to penetrate the filter if drawn through whereas no liquid can penetrate through the Plus filter. Tests with bacteria sample, radioactive solution and plasmid DNA (3) indicate that after 50-100 pipettings contamination can be found in the pipettor barrel when no filters are used. With bacteria samples the pipettor barrel is always maintained uncontaminated when Plus filters are used and in 91 % of the cases when standard filters are used even after 500 pipettings. In cases where the pipettor nose cone, barrel or filter are heavily contaminated with bacteria the carryover contamination is observed in 13 to 20 % of the pipetting series. With the Plus filters the bacteria carryover was not observed. Also with DNA samples carryover was not observed with either filter. If accidental. over-aspiration should happen, carryover occurs irrespective whether there is a filter or not.

Decontamination vs. autoclaving

In nearly all laboratories there is a need for decontamination. Increasing awareness and hygiene regulations make effective use of decontamination a must. As a result, the question arises: to autoclave or to decontaminate. In 99% of cases decontamination is the answer. If we consider general decontamination, using a simple decontamination solution is fast, cost-effective and safe compared to the time-consuming autoclaving procedure. There are non toxic, non irritant, alcohol-free decontamination solutions on the market today, like the Biohit Proline Biocontrol (Fig. 4). This solution is lethal to a broad spectrum of viruses, bacteria and fungi and is ready for use. It can be used simply by spraying the pipettor or the environment with the solution and wiping with dry cloth. Actually this should be done daily in all laboratories to increase safety. If more complete decontamination must be done for pipettors (in case of overaspiration, incorrect pipetting technique etc.), the pipettor should be disassembled and the parts of the tip cone area should be soaked in the liquid for e.g. 30 minutes.

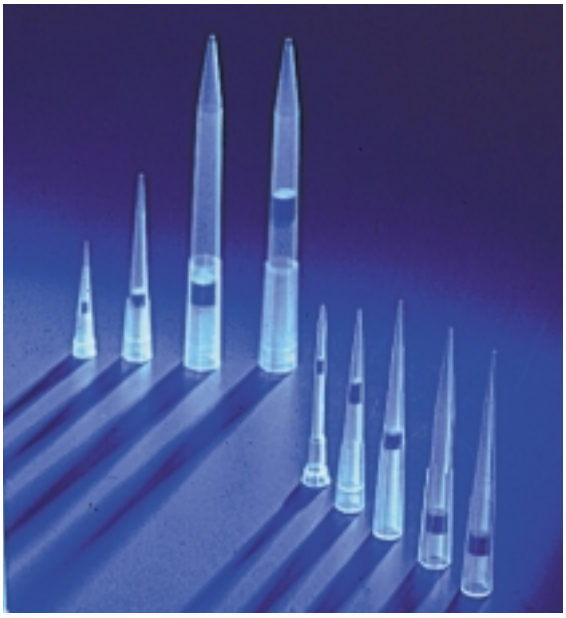

Fig. 2. Filter tips can be used to protect the sample, but are rather expensive.

Fig. 3. The filtered tip cones prevent contamination of the pipettor and carryover to next samples. Forceps that are supplied with every pipettor the filter to be changed easily and cleanly.

Fig. 4. Decontamination is done easily by spraying with specific decontamination solutions, such as the Biohit Proline Biocontrol. For complete decontamination, the tip cone parts can be soaked in the solution for 30 minutes.

Another need in the biotechnology field is to get rid of the RNAse contamination. Even if autoclaving is thought to be effective, it does not fully inactivate RNase. Another possibility is to use DEPC treatment, which is also time-consuming. Moreover, DEPC is suspected to be carcinogenic and must be handled with utmost care, gloves on under fume hoods. However, there are decontamination solutions on the market for this purpose also, for example the Biohit RNase Zap.

Similarly to Biohit Proline Biocontrol, it works by spraying directly to the work surfaces, benchtops, glassware, and pipettors. For complete removal of RNAse contamination of pipettors, the ejector collar should be disassembled and the tip cone assembly should be wiped with the RNase Zap solution (Fig. 5). Because hands are a major source of RNase, one should always keep gloves on when performing RNase decontamination.

Fig. 5. RNase contamination can be removed by specific RNAse zap solution safely, easily and quickly.

Recommendations

1. The user should pay attention to the ergonomics at work (pipettor and environment), take care of the proper shielding and protection (gloves, clothes) and follow good laboratory practise.

2. If no filters are used in an air displacement pipettor its nose cone and barrel have to be cleaned and decontaminated regularly. Only 10 pipettings can already contaminate the nose cone.

3. The filters protect well the internal mechanism of the pipettors but they need to be changed regularly. The interval of changing the filter depends completely on the application and the sample. However, according to studies above the filter is recommended to be changed daily (50-250 pipettings) and always in case of over-aspiration. To ensure the safety of the user, forceps (Fig. 3) should be used to avoid touching the dirty filters by hand. In addition, the tip cone should be cleaned regularly.

4. It is recommended to use standard filters for general applications and the Plus filter for more demanding applications such as cell culture, bacterial and virological work and molecular biology. Standard filters can be used also for this type of work, but they need to be changed more frequently.

5. Cleaning and quick decontamination (spraying) of the pipettors should be done daily. If a serious over-aspiration occurs the nose cone, barrel and piston of the pipettor must to be cleaned and decontaminated immediately.

References

1. David, G. And Buckle, P. A questionnaire of the ergonomic problems associated with pipettes and their usage with specific reference to work-related upper limb disorders. *Applied Ergonomics*, Vol. 28, No. 4, pp 257-262, 1997.

2. Björkstén, M. G., Almby, B. and Jansson, E. S. Hand and shoulder ailments among laboratory technicians using modern plunger-operated pipettes. *Applied Ergonomics*, Vol. 25, No. 2, pp 88-94, 1994.

3. Kolari, M., Mannonen, S., Takala, T., Saris, P., Suovaniemi, O. and Salkinoja-Salonen, M. The effect of filters on aseptic pipetting lifetime of mechanical and electronic pipettors and carryover during pipetting. Lett. *Appl. Microbiol*. 29, 123-129, 1999.

S. Mannonen is Marketing Manager and K. Syrjä Product Manager, Biohit Plc., Helsinki, Finland

Published in *International Labmate*, February 2000 and Lab Asia, March 2000.