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Appraisal of the practical effectiveness of biosafety controls in biotechnology with special reference to safety cabinets

Ray P. Clark

Thermal Biology Research Unit, Kings College London, Campden Hill Road, London W8 7AH

Ray@medi.demon.co.uk

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SUMMARY

This paper describes the development of microbiological safety cabinets for the safe handling of potentially hazardous material. The test methods required to establish their performance are discussed. It is suggested that a similar approach based on Operator Protection Factors could be adopted for other equipment used for biotechnology.

INTRODUCTION

There is worldwide awareness of the need to control the generation and spread of potentially hazardous biological aerosols both in research laboratories and in manufacturing facilities. Arising from this is the need for containment systems and for methodology which can validate their effectiveness in protecting the safety of people and the environment.
Many countries now have legislation which requires that risk assessment be undertaken for work involving potentially hazardous material and that, where appropriate, control measures be provided to prevent exposure to potentially hazardous organisms or toxic material. For example, in the UK, there are the COSHH (Control of Substances Hazardous to Health) Regulations which were introduced in 1988 (4). These provide a framework for the assessment of hazard in relation to appropriate control measures, and back up the requirements of the 1974 Health and Safety at Work Act (1). These COSHH regulations are used to implement the requirements of the EC Directive 90/679/EEC on the protection of workers from biological agents (6).

HISTORICAL PERSPECTIVE

Current concepts for the safe handling of microorganisms date back just over 20 years and, in the UK, were given impetus from the escape of smallpox virus in 1973 and 1978 from laboratories in London and Birmingham. In 1975 a working party set up as a result of the first smallpox outbreak, produced a framework and code of practice for handling potentially pathogenic organisms. Subsequently, there was great interest in improving laboratory safety by developing safe working methods and designing equipment which was suitable for handling pathogenic organisms.

MICROBIOLOGICAL SAFETY CABINETS

In the 1950s the Public Health Laboratory Service (PHLS) in the UK had recognised that special facilities were required to handle pathogenic organisms. This recognition resulted in the design of a Class I microbiological safety cabinet which has remained relatively unchanged in concept to the present day. The Class I safety cabinet is basically an enclosure with air flow from the laboratory, through the front aperture and over the work away from the operator, with the discharge air being filtered. This cabinet was designed specifically, and only, to protect the operator from exposure to any bioaerosols generated within the working area. However, as cell culture techniques became more widely used, there was not only a need to protect the operator from exposure to bioaerosols but also a requirement to protect the work from contamination from any organisms in the general laboratory air. The Class II safety cabinet was developed which enabled both operator and product protection to be available in an open fronted cabinet. Such cabinets became increasingly popular and are now the main kind used in biological research.

For situations where a more rigorous barrier was required between the work and worker, the Class III safety cabinet was developed. This is sometimes called a glove box and the operator works through gloves mechanically attached to the cabinet which itself is maintained at negative pressure by a flow of air which is filtered as it enters and leaves the cabinet. Glove boxes were also extensively used in the nuclear industry and sophisticated designs evolved. Test methods for glove boxes were also developed and the technology
became well developed. Thus, during the late 1970s microbiological safety cabinets in three classes (I, II and III) were becoming established and widely used.

**STANDARDISATION**

In some institutions specially designed facilities were built specifically to handle highly pathogenic organisms such as smallpox and there were regular and rigorous checks of the filters and seals and glove systems to ensure their integrity. However, in hospitals, research institutes and universities many safety cabinets were used with little regard to the real effectiveness of the systems. Problems of performance were much more common with cabinets having an open front and relying for their containment on an air curtain at the front aperture. Although open fronted safety cabinets came into widespread use in the mid 1970s there were very few methods available to test their effectiveness.

There was clearly a need for standardisation of some kind and in 1976 the *National Sanitation Foundation of America* published NSF 49 which specified tests for filters and seals and also described a test to evaluate the effectiveness of the air curtain at the front of *Class II* cabinets. This standard was followed in 1979 by the publication of *British Standard 5726 (3)* which, introduced the concept of a quantifiable "Operator Protection Factor". The protection factor concept is due to Dr. Owen Lidwell (9) who chaired the *British Standards Institute* committee which published the 1979 safety cabinet document.

It was recognised that the containment performance of safety cabinets could not be defined simply by air velocity; effective containment was a function of air velocity, turbulence, internal geometry and aerodynamics as well as external influences in the immediate environment, such as the movement of people and doors, proximity of ventilation ducts etc. It was realised that the only realistic way to test for the level of containment was to mimic a work situation where aerosol was released inside the cabinet and to identify and measure that which escaped from the cabinet. Here the Operator Protection Factor proved to be of real value.

The original way of measuring operator protection was to release an aqueous bacterial aerosol within the safety cabinet and to use strategically placed bacterial samplers in front of the working aperture to quantify escape of organisms. The *Operator Protection Factor* numerically describes the results of such tests in a way that relates the exposure resulting from the escape of hazardous aerosol from the cabinet to the exposure that a worker would experience if the work was carried out on the open bench. It specifies the results of tests in a non dimensional way which does not rely on any particular test method. It can be applied to tests other than microbiological, as long as the test system meets various criteria concerned with aerosol generation, sampling and identification.

The 1979 version of *BS 5726 (3)* required manufacturers to submit examples of *Class I* and
II safety cabinets for validation tests to show that they achieved Operator Protection Factors in excess of 10^5. This rapidly brought about improvements in the cabinets available. However, it became apparent that although a particular safety cabinet may have passed the validation test, this did not guarantee that others in a production run would perform in exactly the same way. Subtle variation of manufacturing technique or tolerances, of fan and filter specifications and of the environment in which the cabinet was put, could dominate performance.

It therefore became important to test individual cabinet installations at commissioning and subsequently at routine maintenance periods. Clearly it was not desirable that these on-site tests used a challenge aerosol of microorganisms, such as *Bacillus subtilis* spores or pigmented cells of *Serratia marcescens*, which were commonly used initially. Use of these could cause gross contamination of cabinets and laboratories (some 10^8 cells would be released in a single test) and there was also a potential health hazard. *Serratia marcescens* is a low grade pathogen (Ref) and has been responsible for bacteraemic infections, while some individuals are allergic to *B. subtilis*. For all of these reasons the use of microbiological tests was, and is, restricted.

To meet the new need for an *in-situ* test the potassium iodide KI Discus system was developed (8) which enabled on-site measurements of containment to be made whenever safety cabinets were commissioned and maintained. This system produced a standard aerosol from an aqueous solution of potassium iodide (8).

In the period between 1979-1992 the concept of measuring Operator Protection Factor at the work place became widely accepted and the subject of regulatory requirements in the UK and elsewhere. In consequence, the latest version of BS 5726, published in 1992 (7), now specifies in considerable detail the KI test system and how it is to be used for validation, commissioning and maintenance tests.

**WIDER USE OF CONTAINMENT TESTS BASED ON THE OPERATOR PROTECTION FACTOR**

During the period of the development of tests for safety cabinets, the same tests were frequently applied to laboratory fume cupboards and to other local exhaust ventilation systems where operator protection needed to quantified. Such measurement techniques were, and are still, in advance of tests for containment or leakage from fume cupboards which are based on trace gas analysis. (5)

The application of the concept of Operator Protection Factor and its measurement using potassium iodide was extended to the containment measurement of complete laboratories or suites of laboratories. If a potassium iodide aerosol is liberated within a room then aerosol that escapes into the surrounding areas can be measured and it is possible to define
an Operator Protection Factor between the room and the surroundings.

There is considerable merit in considering a single, unifying philosophy and methodology for containment measurement over a wide range of open fronted local exhaust ventilation systems (safety cabinet, fume cupboards, local hood ventilation systems, carcinogen and radio pharmaceutical safety cabinets). The concept would seem to be of potential value in quantifying containment in large scale biotechnology plant.

ARE CURRENT CONTAINMENT TESTS ADEQUATE?

For open fronted safety cabinets used for handling potentially pathogenic organisms, the fact that some of a test aerosol escapes from the safety cabinet working area into the laboratory, demonstrates that the containment of such cabinets is not infinite; nevertheless, the requirement that the Operator Protection Factor should be greater than $10^5$ has proved to be a realistic value for high protection. However, the process contemplated may require a greater level of containment than is possible from a safety cabinet. Complete containment in cases where any contact of operators with organisms is undesirable will require the segregation of the worker from the work with all of the operational difficulties that this entails.

An important point about the use of the containment test is that it can instantly identify those cabinets which perform badly due to design or installation/operation fault or because of a poor environment.

APPLICATION OF SAFETY CABINET CONCEPTS

From an equipment and testing standpoint, safety cabinet evolution and the development of Operator Protection Factor tests is very much a success story. The methodology is now well established and is now spilling over into other areas of science where containment is important (such as fume cupboards etc.). Another important principle and one reason why safety cabinet standardisation work has been so successful is that the definition of performance criteria does not take into account the potential hazard of the materials that are to be used in the cabinet. The criterion is the performance of the cabinet. British Standard 5726 (7) gives guidance on construction and testing procedures whereby a cabinet can perform to a consistent, measurable, and sustainable level. The methods of test give easy comparison between individual cabinets at different times and between different cabinets and allows then to be used with confidence to provide the defined level of protection.

Protection of the worker requires risk assessment. The value of the Operator Protection Factor is that it allows risk assessment to be carried out on the basis of reliable quantitative information. It is only when this is available that an objective risk assessment can be made.
as to whether the performance of the cabinet is satisfactory for the work proposed having
due regard to the pathogenicity of the organisms involved.

Such an approach, which defines performance and allows any legitimate and justifiable
method of defining it, allows different, and possibly innovative, methods of achieving a
given and specified level of performance.

**BIOTECHNOLOGY STANDARDISATION**

Can the principles and methods adopted for safety cabinet standardisation be applied for
other equipment where there is a need to protect operators, workers and the environment
from exposure to organisms leaking from equipment and processes?

This question is particularly important in relation the CEN Standards initiative for
Biotechnology which aims to produce 55 standards in the whole field of biotechnology
and, in particular, 21 standards for the performance of individual types of equipment.

The standards for performance criteria will not, except in specific cases, address questions
of product quality, since there are many standards and codes of practice already dealing
with this. They will be concerned with criteria for protecting workers and the environment
from any organisms which may be potentially hazardous. The concept of defining the
performance and then, separately, deciding whether performance of the equipment with a
given level of performance is adequate to control the risks inherent in a particular process,
seems to be generally appropriate for standardisation of biotechnology equipment. In many
cases the difficulty experienced initially with safety cabinets - that there are inadequate
testing methods - will apply to other items of equipment and create the need for research to
develop such methods.

**REFERENCES**


2 Anon., 1976. US National Sanitation Foundation Standard No.49, *Class II* (Laminar-
flow) Biohazard Cabinetry.

3 Anon., 1979. British Standard BS 5726, British Standards Institution, Linford Wood,
Milton Keynes, MK14, 6LE, UK.

0 11 086757 1 and Approved Codes of Practice (HMSO-ISBN 0 11 885468 2).

5 Anon., 1990. British Standard BS 7258: Laboratory Fume Cupboards, British Standards
Institution, Linford Wood, Milton Keynes, MK14, 6LE, UK.


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