THE CLEANROOM MONITOR

S2C2

The Scottish Society for Contamination Control

March 2004 **Issue 49**

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NEW ISOLATOR BOOKS COMING OUT IN MAY 2004

ISOLATION TECHNOLOGY: A PRACTICAL GUIDE
2nd Edition
Tim Coles, Cambridge, UK
Published by CRC Press 232 pages.
ISBN: 0 849319447
Cost £137.00 ($199.95) *See page 10.*

PHARMACEUTICAL ISOLATORS
A GUIDE TO THEIR APPLICATION, DESIGN AND CONTROL
1st edition *
Edited by: Brian Midcalf, W Mitchell Phillips,
John S Neiger and Tim P Coles.
Published by Pharmapress 272 pages. Cost £29.95
Paperback. ISBN: 0 853695733.
** This is the new edition of the Yellow Guide* *See page 10.*

Expression of Isolator Leak Rate - Percentage Volume Change per Hour

A Guide for Normal People

Tim Coles and John Neiger* Tim and John are also editors with Brian Midcalf of the new Yellow Book "Pharmaceutical Isolators" which is due to be published in May.

Part A Introduction

Isolators are frequently leak tested by a test known as the pressure decay test. It's not a perfect method, but it is simple and practical.

Pressure decay may be reported in a variety of forms but the most common, because it is the easiest to grasp, is as **Percentage Volume Change per Hour**. This volume change is actually a volume loss in positive pressure isolators and a volume gain in negative pressure isolators. The **Percentage Volume Change per Hour** is the volume of air leaked out of or into the isolator during the period of the test, expressed as a percentage of the total volume of the isolator per hour or **$\%h^{-1}$**.

The disadvantage of **$\%h^{-1}$** is that percentages do not lend themselves to arithmetic as readily as absolute values.
It is probably for this reason that "hourly leak rate" in units of **h^{-1}** is used in ISO10648-2 and BS EN ISO 14644-7. This paper uses both expressions.

Leak testing and expression of leak rate are described in detail in the new "Yellow Guide" - "Pharmaceutical Isolators" due to be published by the Pharmaceutical Press in May 2004. [see page 10]. The guide points out that isolator manufacturers should specify appropriate leak rates for the application or process.

Three classes of isolator are promulgated in the ISO standards based on a starting pressure differential of 1000 Pa (Pascals) for acceptance tests and 250 Pa for operational use checking:

Class I Isolators	$\%h^{-1}$ = less than 0.05% h^{-1} = less than 5×10^{-4}	This level is used for high containment enclosures such as nuclear gloveboxes and was the level for Class III Microbiological Safety Cabinets in BS 5726: 1992, but BS EN 12469: 2000 specifies less than 0.10% from a starting pressure of 500 Pa.
Class 2 Isolators	$\%h^{-1}$ = less than 0.25 % h^{-1} = less than 2.5×10^{-3}	This level may be used for turbulent flow isolators with a low air change rate of around 180 air changes per hour. It's a good standard for large pharmaceutical isolators.
Class 3 Isolators	$\%h^{-1}$ = less than 1.0% h^{-1} = less than 1×10^{-2}	This level may be used for smaller non-gassed positive pressure isolators and negative pressure laminar flow isolators.

Whatever the class of isolator, it is important to note that if it is gassed, then the acceptable leak rate must give an acceptable OEL for the gas outside the isolator.

The average isolator user running a pressure decay leak test will want to know either:

1. If the starting pressure differential, the pressure change and the time of a pressure decay test are measured, how do you calculate the Percentage Volume Change per Hour, the Hourly Leak Rate or The Class of Isolator?

or:

2. If the Class of Isolator, the starting pressure differential and the time are defined, what is the maximum acceptable pressure change in a pressure decay test?

Or perhaps:

3. If the Class of Isolator and the pressure change are defined, what is the minimum time allowed for that pressure change?

Part B. Calculating the Answers to These Questions

In all these calculations, you need to use absolute pressure, expressed in Pascals (Pa). Atmospheric pressure is roughly 100,000 Pa so just add this to your figures for starting pressure or end pressure. Typically, most people will probably have a starting pressure of 100,250 Pa. Real anoraks will actually measure the atmospheric pressure at the time of the test and use this value for an accurate answer, but normal people need not bother - though be aware of Part C of this work. The test time needs to be in minutes. As the expression uses a percentage of the volume of the isolator it is not necessary to know the actual volume for these calculations.

1. Calculate the $\%h^{-1}$ or h^{-1} (and thus find the class of isolator)

$$h^{-1} = \frac{\text{starting pressure - end pressure}}{\text{end pressure}} \times \frac{60}{\text{test time mins}}$$

$$\%h^{-1} = 100 \times h^{-1}$$

2. Calculate the maximum acceptable pressure change (starting pressure - end pressure)

You need to enter the class of isolator (as h^{-1}) in these calculations as follows:

For Class 1: enter 5×10^{-4}
For Class 2: enter 2.5×10^{-3}
For Class 3: enter 1×10^{-2}

$$\text{Maximum acceptable pressure change} = h^{-1} \times \frac{\text{test time mins}}{60} \times \text{end pressure}$$

The maximum acceptable pressure change is the maximum pressure change in the test time for the isolator to pass the test for the given class.

3. Calculate the minimum time allowed for a given pressure change

You need to enter the class of isolator (as h^{-1}) in this calculation as follows:

For Class 1: enter 5×10^{-4}
For Class 2: enter 2.5×10^{-3}
For Class 3: enter 1×10^{-2}

$$\text{Minimum time for given pressure change} = \frac{\text{Pressure change}}{\text{end pressure}} \times \frac{60}{h^{-1}}$$

Part C. Other Things You Need to Know

1. Test Pressure

The value for **$\%h^{-1}$ or h^{-1}** that you get is only correct at the test pressure you used. If you try another test pressure, you will get a different value, so you need to state clearly what pressure (the start pressure) was used for the test.

What sort of test pressure should you use? Well, recommendations vary from between 1.5 times working pressure to 1000 Pascals. Ask the isolator manufacturers what they recommend. Be wary of over-pressuring rigid wall isolators: you can easily crack a window.

2. Linearity

The above calculations assume linearity of leakage over the time of the test, which is not strictly correct - clearly the isolator will leak more when the pressure differential is higher at the start of the test than at the end of the test when it is lower. Well, we could go into all sorts of complex numbers (like “e”) but in practice, these calculations are just fine for normal people.

3. Correcting for Temperature and Pressure

All these calculations are based on the Universal Gas Law, which is:

$$\frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2}$$

P = absolute pressure (in Pa)
V = volume (in m^3)
T = Temperature (in degrees C)

Generally speaking, temperature is ignored and so is atmospheric pressure, but if you want really supportable data or you are working with a Class 1 isolator, then you need to consider:

a. Changes in the internal temperature of the isolator during the leak test

When the temperature in the isolator goes up during the test, the pressure will rise. For every 1^{o}C temperature increase, the pressure will rise by about 340 Pa, which is quite a lot.

If you measure the internal temperature of the isolator (not very easy to get an accurate representative value) you can correct for temperature changes as follows:

Use a two-decimal place digital thermometer inside the isolator. If the temperature has gone up, subtract 3.5 Pa from the end pressure figure for every 0.01^{o}C rise. If it goes down, then add 3.5 Pa. This is true also for negative pressure tests, which means if the temperature rises, you make a negative end pressure reading more negative.

If the temperature does not change at all, then you have an adiabatic test. You can use this word to impress people at parties.

b. Changes in atmospheric pressure during the leak test

When the atmospheric pressure rises during the test, the pressure in the isolator appears to go down. For every 1 mb of atmospheric pressure rise, the isolator pressure will appear to drop by 100 Pa - still quite lot. Given that atmospheric pressure can change by several mb in an hour, you may well want to correct for this.

Use a precise barometer reading to 0.01 mb or 1 Pa. If the pressure has gone up, add 1 Pa to the end pressure figure for every 0.01 mb (or 1 Pa) rise. If it goes down, subtract 1 Pa. Again the same is true for negative pressure tests - if the pressure rises, you make a negative end pressure reading less negative.

Once you have corrected the end pressure reading, you can use the figure in the calculations for $\%h^{-1}$ or h^{-1} as above.

4. Sleeves and Suits

Isolator sleeves can make a mess of leak tests and half suits can make a real mess of them. The problem is that they move about and change volume during the test - and a 0.10% change in the isolator volume will change the pressure by 100 Pa.

In positive pressure tests, evert (a posh word for “pull out”) the sleeves and gloves fully. The inflated sleeves will act as pressure compensators to some extent but at least they will not change volume. In negative tests, try to get the sleeves inflated into a “relaxed position” inside the isolator - not easy in most isolators.

Some users test the sleeves and gloves individually, at frequent intervals, using one of the proprietary devices available from isolator manufacturers. Since gloves and sleeves are the most likely source of leaks, this is a good idea. You can apply the same criteria to these tests as the full isolator test and allocate a class to them.

Half-suits are bit more of a problem to test on the isolator because they move about even more than sleeves. In positive pressure isolators, the best plan is to inflate the isolator to test pressure, gently shake the suit down into a relaxed position, and then re-inflate the isolator again. In negative pressure isolators, half-suits inflate rather dramatically into the isolator but can still be gently shaken to try and get them into a stable “relaxed position”. This may take a

bit of practice, but you will eventually get reproducible results. If your suit is suspended by elastic cords, replace them with rigid cords or the suit will act as an efficient pressure maintaining device.

Part D. What to do When it Leaks

All isolators leak but if your isolator has a leak rate that is more than is acceptable for the class, you need to find the holes and then fix them. One method for finding leaks is to use helium gas and a "sniffer". Connect your helium cylinder (balloon gas is fine) to the isolator, close all the valves and take the isolator to a suitable test pressure (between 1.5 and 5 times working pressure) using the helium. This will give enough helium concentration for your sniffer to detect - try cracking open a port with the sniffer at hand to check. Take care not to over-pressure the isolator as this can spoil your whole day.

Now go over every seal and joint in the system with the sniffer. Re-inflate with helium from time to time. It can take some time but eventually you will track down the leaks. Don't forget that some of the ductwork may form part of your sealed enclosure so all of this needs a good sniffing too. This all gets easier with experience.

Another method is to use DOP instead of Helium as the challenge. DOP is not as searching as Helium and does not pass through HEPA filters, therefore every chamber has to be done separately, but the advantage is that most test engineers already carry DOP equipment for testing HEPA filters.

How you fix any leak depends on the nature of the leak, but try not to paste everything with silicone rubber. You may have to dismantle some things, clean them and reassemble more carefully than the last person did.

So, that's it! Now you can leak test your isolator by pressure decay and report the results with confidence.

* BIOGRAPHICAL NOTES

Tim Coles:

Tim Coles is an Engineering Specialist and has been working for GRC Consultants, a division of the Mott MacDonald Group, since 1998.

His main interest has been in pharmaceutical isolation technology, particularly in the field of aseptic operations and gas phase sanitisation. This work has included the design of facilities, specification of the equipment and subsequent validation.

Tim holds B.Sc. and M.Phil. degrees in Environmental Sciences from the University of East Anglia. He has previously worked for equipment manufacturers including La Calhene GB and his own company, Cambridge Isolation Technology Ltd.

His book, *Isolation Technology - A Practical Guide* was published by Interpharm Press Inc (now CRC Press) in 1998 with a second edition (ISBN: 0849319447) due out in May 2004. It is 232 pages and costs £137.00 ($199.95).

Tim writes and lectures regularly on the subject of isolation technology.

John Neiger:

John Neiger was a founding director of Envair Limited in 1972 and is currently Chairman. In 1986 Envair entered the field of isolators and, in collaboration with the Royal Hallamshire Hospital, Sheffield and Hope Hospital, Salford, developed some of the original hospital pharmacy isolators. Shortly after the UK Pharmaceutical Isolator Working Party was set up by the NHS Regional QC Pharmacists Committee in 1993, he became a member and has remained so ever since.

He sits on the BSI committee responsible for UK input into the ISO 14644 "Cleanrooms and associated controlled environments" series of standards and is one of the UK technical experts on the ISO Working Group that drafted the part concerned with Isolators, Part 7: Separative devices (clean air hoods, gloveboxes, isolators, minienvironments). He also sits on the BSI committee which is responsible for the UK contribution to EN 12469 "Biotechnology - Performance Criteria for Microbiological Safety Cabinets". He is a committee member of the Contamination Control Group of the Society of Environmental Engineers.

ISO 14644-8 WG8 AIRBORNE MOLECULAR CONTAMINATION

Mr David Clough, Convenor, ISO/TC 209 Working Group 8 states "A major new area of emerging interest in airborne molecular contamination (AMC) appears to be in the Biotechnology and Life Science Industries. Cleanroom AMC control will be required for research and production facilities in such areas as DNA-Chips, gene chips and IVF laboratories.

Care has to be taken in monitoring AMC's to include only gaseous contaminants and exclude particulate contaminants. For example Boron can be found as a particulate and as a gas.

Clients can be confused and ask for AMC monitoring which includes particulate contaminants such as aluminium, copper, sodium, etc."

Report by Mr David Clough, Convenor, ISO/TC 209 Working Group 8.

ISO 14644-8

This part of ISO 14644 will assign ISO classification levels to be used to specify the limits of airborne molecular contamination, (AMC) concentrations within a cleanroom and associated controlled environments where the product or process is deemed to be at risk from such contamination. The standard will be generic and designed to be used by a wide range of industries within which AMC's have a deleterious effect.

Molecular contamination is a three step event.

The first step is *generation* due to external sources, process leakage or (construction) material outgassing.

The second step is *transport* as AMC in air.

The third step is *sorption* on the sensitive surface which can be quantified as a surface molecular contamination (SMC).

Key points of the draft include

* Airborne molecular contamination is defined as the "presence in the atmosphere of a cleanroom or controlled environment of molecular (non-particulate) species in the gaseous or vapour state, which may have a deleterious effect on the product, process or equipment in the cleanroom or controlled environment";
* quantitative classification of clean-rooms and associated controlled environments according to the level of contamination;
* flexible contaminant categories within which to group AMC's, e.g. biotoxics, corrosives, bases, organics, etc.;
* informative examples of sampling strategies and analysis methods;
* frame work for the unambiguous reporting of results used for classification;
* detailed examples of specific AMC's and how they can be classified using the standard;
* the use of a negative log scale for the units of measurement, (g/m3) which is related to classification class within the envelope of current technology, but will allow further expansion as technology and user requirements develop;
* provision of a bibliography;
* designed to be used by a wide range of industries, including pharmaceutical, medical, aerospace, semiconductor, food, and nuclear processes.

The new standard currently confines itself to atmospheric contamination, thereby excluding the specific consideration of contaminants deposited on materials or equipment in a clean environment. There is a proposal currently before ISO TC209 for a separate standard for SMC's (surface molecular contamination).

Documentation

Any documentation for consideration by ISO/TC 209/WG 8 may be submitted, via IEST or one of the national standards bodies that are members of ISO/TC 209, to either:

David Clough, Convenor, WG 8,

Envirotest Ltd, Unit 9 Pond Close, Walkern Road, Stevenage, SG1 3QP. Tel: +44 (0)1438 361112

or

David Michael, BSI contact, WG 8.

BSI Standards, 389 Chiswick High Road, London, W4 4AL

+44 (0)20 8996 7219

PROFILE - Willis Whitfield

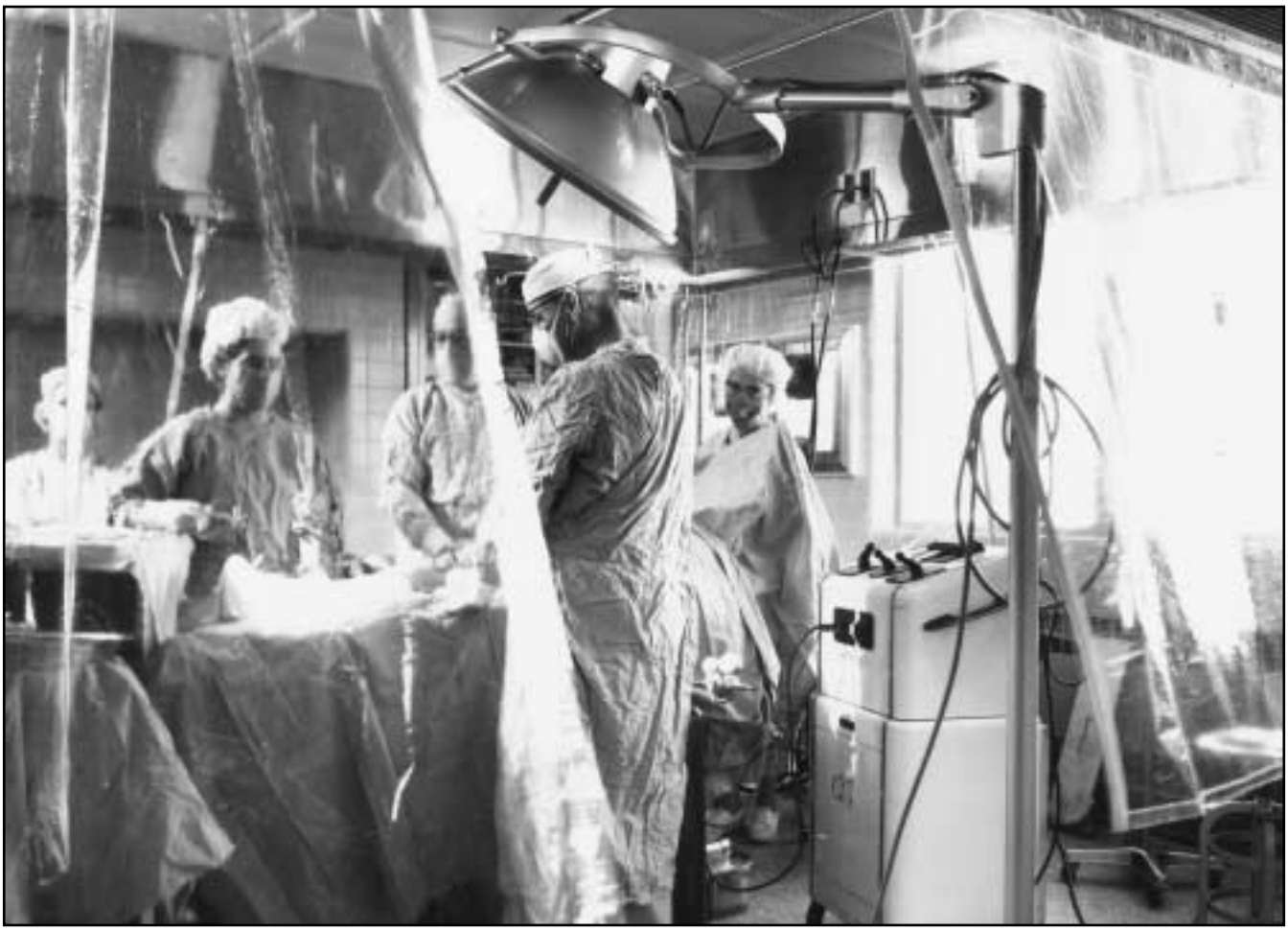
Willis Whitfield, based in Sandia Laboratories, Albuquerque, New Mexico, USA invented and developed laminar airflow devices for military and then industrial use in the early 1960s. [See Issues 47 and 48]. Very quickly these new "cleanrooms" attracted the attention of the the medical establishment.

The proximity of Sandia Corporation was one of the reasons Dr John G Whitcomb, a surgeon based in Bataan Hospital in Albuquerque wanted to explore the idea of installing a cleanroom in his hospital.

Infection in hospitals had always been a long-standing problem. (In 1936 Dr Frank Meleney compiled a list of 6 causes of infection; airborne bacteria ranked number 4.) While antibiotics started to be used for civilian population after their development for the military in the Second World War, there had been no similar major dramatic advancement until the medical people, looking for a solution, turned to the idea of using HEPA filtered air.

The concept was discussed with Willis Whitfield, J Gordon King, Bill Soltis of Comfort Air Service (who built it), Wm Randolph Lovelace and James Goddard (FDA, also physician and engineer). Goddard had seen a portable, curtained downflow unit which the Air Force had used. "Dr Goddard was quick to point out that this was not true laminar flow but was really controlled isotropic turbulence." [5] Initial planning began in 1963 and the design was completed and installed in 1964. It was in regular operation from 1966.

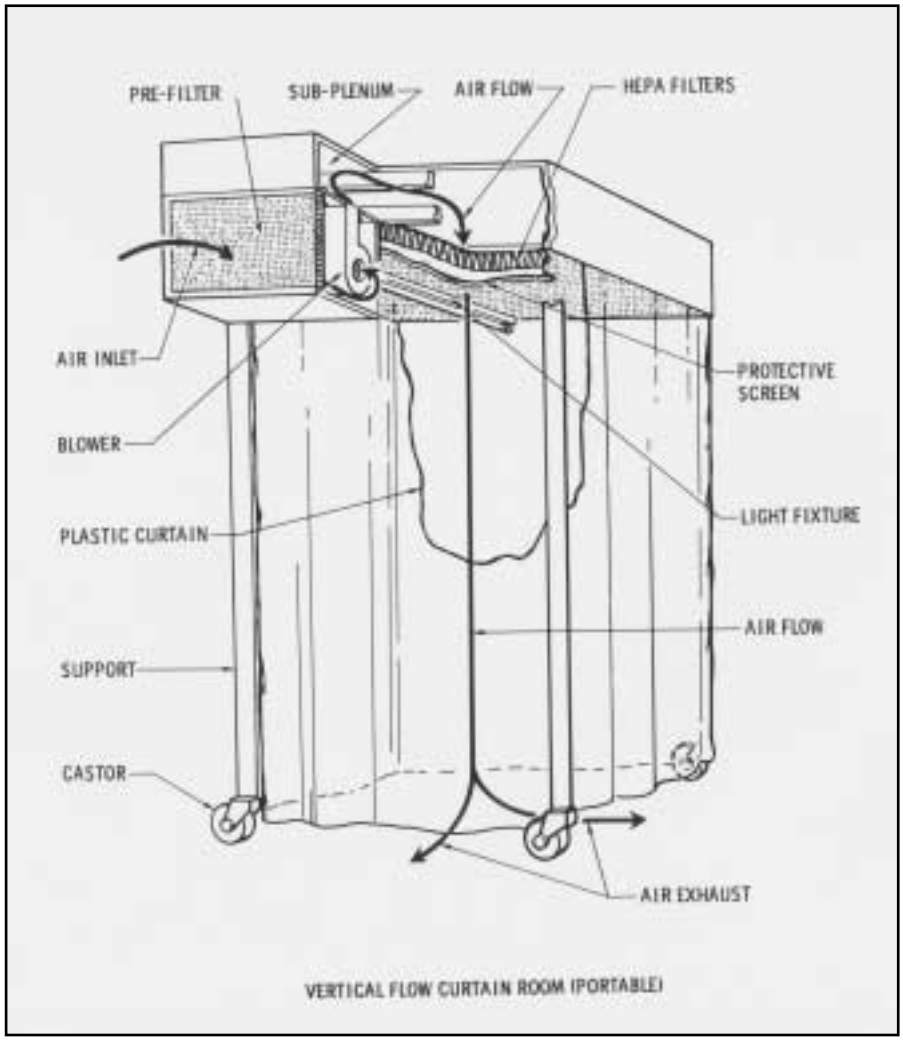
The first microbiological experiments were performed in a laminar flow room at Sandia Laboratory in early spring 1962 and the contamination shown by the settling samples was



Vertical Flow Curtain Room (Portable) This is the first laminar flow operating facility in the world. It had plastic curtains at the side and used vertical laminar flow from HEPA filters suspended from the ceiling above the operating table. Six blowers provided vertical air flow of 90 feet per minute or about 600 air changes an hour. Details of its design, construction and testing can be found in Reference 5.

Part 3: HOSPITALS

significantly lower than that expected in modern surgical facilities. For example, in one operation bacterial counts beside the operating table dropped from an average of 6 to 7 colony forming units per cubic foot of air to less than 0.5 organisms per cubic foot within two minutes of starting the filter unit. This new system was so dramatically effective in removing airborne bacteria that Whitfield writing in 1967 could see that his invention "opens a new era for clean room use in many hospital situations [and that] such systems should be beneficial in the pharmaceutical field." [2]



VERTICAL FLOW CURTAIN ROOM (PORTABLE)

Speaking from Albuquerque today, Willis recalls the people involved: "This reminded me of another far-reaching aspect of laminar flow clean room usage. When Dr. Goddard was the head of the FDA, he spent a half-day with me in Albuquerque (at Sandia) looking for an answer to the Penicillin Contamination problem in pharmaceutical manufacturing plants in the U.S. After seeing demonstrations in the original clean room (Laminar Flow) and data, he returned to Washington and issued a directive to all pharmaceutical companies to conduct all exposed manufacturing operations in laminar flow hoods or clean rooms - immediately. This enabled the manufacturers to stop the Penicillin cross-contamination problems."

The origin of the term "laminar flow"

Willis states: "You probably have not heard how the 'Laminar Flow' name got attached to that type device. The first manufacturer, The Agnew Higgins Co. attached 'Laminar Flow' to his cleanroom products and as a result, the name came into common use. The name became official with the U.S. Government when Federal Standard 209 was completed. It was Mr. Tom Casberg's (GSA) idea to define it in the standard to specifically identify laminar flow devices."

Photos and diagram photo taken by Sandia Corporation and kindly supplied by Willis Whitfield.

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1. McDade, JJ; Phillips, GB; Sivinski, HD and Whitfield, WJ. Principles and Applications of Laminar-flow Devices, *Methods in Microbiology*, (Eds. Norris, J & Ribbons, D), Academic Press Inc (London) Ltd, Volume 1, Chapter V, 1969, pages 137-168.
2. Whitfield, WJ. Microbiological Studies of Laminar Flow Rooms, *Bulletin of the Parenteral Drug Association*, Parenteral Drug Association, Inc, Vol 21, No 2, 1967, page 49.
3. Coriell, LL and McGarrity, GJ. Elimination of Airborne Bacteria in the Laboratory and Operating Room, *Bulletin of the Parenteral Drug Association*, Parenteral Drug Association, Inc, Vol 21, No 2, 1967, pages 46-54.
4. Bechtol, CO. The Use of Total Vertical Laminar Air Systems in Surgery, *Contamination Control*, A2C2, Blackwent Publishing Company, Inc., California, Vol X, No 9 & 10 Sept/Oct, 1971, pages 18-22.
5. Garst, DM. The Role of Laminar Airflow in Hospitals: A Case History, *Contamination Control*, A2C2, Blackwent Publishing Company, Inc, California, Vol X, No 9 & 10 Sept/Oct, 1971, page 14.

THE YELLOW GUIDE - COLES' COMMENTS

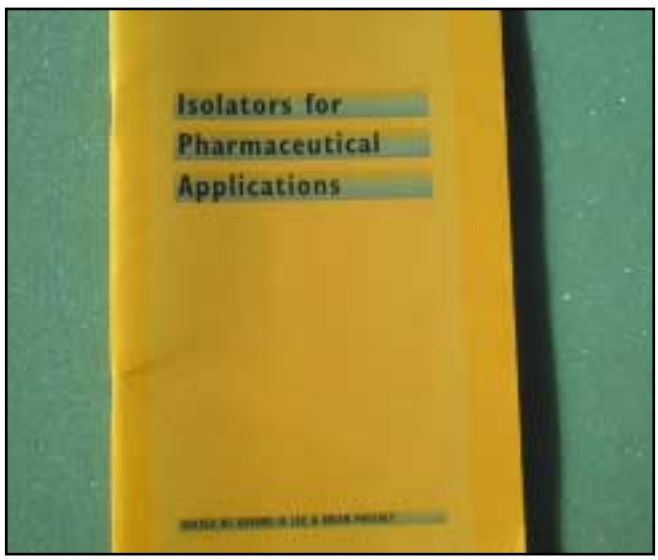
TIM:

Caroline and Tim Coles

The background to the *Yellow Guide* started with hospitals. It goes back to the UK Pharmaceutical Isolator Group, now part of Regional Health Authority Quality Assurance Group. The hospital QA people decided away back in distant time when they were first starting to use isolators that something had to be done because they had no standards or guidelines. Indeed there was a disaster in Manchester, called "The Manchester Incident" in 1992. An isolator was being used to prepare neo-natal TPN (total parenteral nutrition), i.e. intravenous feeding which needs to be sterile because it is being administered intravenously. Therefore it was very important not to introduce anything even mildly pathogenic. Using an isolator was a sensible thing to do but the operators seemed to be under the impression that this was a magical device and somehow, as long as you put it inside the isolator, it would be fine. But, of course, you still must use basic microbiological techniques. You must run aseptic techniques inside your isolator. They didn't. They did not clean up; they did not change things frequently enough and a specific bacterium got into the peristaltic pump tube that was delivering this material and infants died. So this really was the result of haphazard use of an isolator.

Therefore it was really down to lack of training of the operator rather than technology. It shows the dangers of operating equipment if you are not sure of its boundaries, its limitations, its technology.

That galvanized the group and they produced a booklet very quickly, i.e. a booklet that was purely for the NHS. Then it was expanded a bit and the booklet "Isolators for Pharmaceutical Applications" edited by Gerard M Lee and Brian Midcalf was produced (1992) and is commonly known as the *Yellow Guide*.



It quickly became the bible for isolator users but it was pretty limited. We realised this at the time and while there is good information there, it needed expanding and improving. Ever since it came out we have been working on the next version. It has taken a very long time, much longer than we ever thought!

John Neiger spent a great deal of time on this. Brian, John and I got together every Thursday night at John's house in Manchester. First John would take us to a Thai restaurant for a jolly good meal and then we would work until dead of night. Then I would drive back to my hotel in Wrexham which they had locked and I couldn't get in!

The second edition of my book is based on the information generated, i.e. the digested information that we collated. I have said in the forward of my book that I owe a lot to the *Yellow Guide* because I trawled through it looking for information on standards and so forth.

There is a whole chapter on standards. Nowadays, there have not been big changes in technology but the area that people should be updating themselves on is STANDARDS, namely, Chapter 11 Standards and Guidelines.

CAROLINE:

Where this guide started out as a sort of booklet this new edition is going to be around 200 pages. It is beefy and long awaited. It fills in a lot of the gaps with, for example, information on materials used, alternatives. There is a lot of practical information. For example, there is a section on Stainless Steel. You don't need to be a metallurgist or a welding expert but you do need to know the difference between stainless steel 316 and 304, for instance.Or, how do you specify the finish of your isolator? The materials? Does the engineer know what you want? How do you articulate what you want? (found in Appendix 3).

The working party that produced these guides also spawned the Isolator User Group and the Isolator Conferences called the Leeds Conference of which they now have had 6. This is where information gets picked up and incorporated into the *Yellow Guide* and vice-versa. The next Conference is hopefully going to be in December 2004 in Warwick.

(Mike Foster is S2C2's representative on the 14644-7 standards committee who put it together.

ISO 14698 COURSE

ISO 14698 Parts 1 and 2

Cleanrooms and Associated Controlled Environments - Biocontamination

Thursday, March 25, 2004

University of Glasgow

An afternoon meeting to discuss their content

After a wait of many years, ISO 14698-1 and ISO 14698-2 have been published. These standards contain new requirements for cleanrooms where micro-organisms have to be controlled.

Speaking: Bill Whyte and Andrew Tweedie

Members: £105.75 (non-members £123.38)

Standards available. Part 1: £79.20 & Part 2: £48.60

Full details and booking form

Mrs Kay Johnston,
S2C2 office,
James Watt Building, Glasgow University,
Glasgow, G12 8QQ
Tel: 0141 330 3699
Fax: 0141 330 3501
E-mail: s2c2@mech.gla.ac.uk

CTCB COURSE

Cleanroom Testing and Certification Course and Exams

June 22 - 24, 2004

The Erskine Bridge Hotel, near Glasgow

The Cleanroom Testing and Certification Board (CTCB) offer a distance learning course and examination on Cleanroom Testing.

Candidates for both Professional and Associate CTCB certification for Cleanroom Testing must attend a 2 day revision course and examinations. An additional 1 day course given the day before must be attended by Associate candidates.

There will also be an opportunity for others to attend only the lecture on June 23.

Full details and course application forms

Note: The Irish Cleanroom Society will be hosting their next Cleanroom Testing course on November 9 -11, 2004 in Swords, Dublin.

Contact: Peter Fernie, Fernie Technical Services, Tawin Maree, Oranmore, Galway, Ireland. Email: emat@iol.ie

AT YOUR SERVICE

AT YOUR SERVICE