Microbiological monitoring during aseptic handling: Methods, limits and interpretation of results

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ABSTRACT

Aseptic handling is the procedure to enable sterile products to be made ready to administer using closed systems (EU Resolution CM/Res(2016)2). Microbiological monitoring (MM) and media fills are used for environmental and process control.

In this study, the application of MM methods during aseptic handling inside, or related to working in, a laminar airflow cabinet or safety cabinet in hospital pharmacies is described and evaluated. Results are expressed as colony forming units (cfu) and Contamination Recovery Rate (CRR; the rate at which MM samples contain any level of contamination -USP<1116>-). For trend analysis, a rolling CRR is developed (a rolling CRR calculates a CRR using a predetermined number of most recent samples).

Of all MM methods, glove print is the most informative. The added value of air sampling is doubtful. Because of microbiological as well as statistical considerations, the use of CRR for assessing MM results is advised. Glove prints, in general, give the highest CRR. A CRR < 10% is a realistic limit for MM during aseptic handling in hospital pharmacies. A rolling CRR, calculated using the last 100 samples, is a good compromise between reliability of the CRR value and timely prediction of process changes.

1. Introduction

Aseptic handling is the procedure to enable sterile products to be made ready to administer using closed systems (Resolution CM/Res, 2016). The starting materials are sterile and must be kept so during this process (Boom and Beaney, 2015). Aseptic handling itself is performed in a laminar airflow cabinet (LAF), a safety cabinet (SC) or in an isolator (I). The background area is the room where the LAF, SC or I are housed.

In previous articles, the risk sources of non-sterility during aseptic handling were evaluated and measures to keep the risks as low as possible were described (Boom et al., 2020a,b). To verify the effectiveness of these measures, microbiological controls, like microbiological monitoring (MM) and media fills, are important instruments (Boom and Beaney, 2015). In the Netherlands, procedures for these microbiological control instruments are standardised and used in nearly every hospital pharmacy (LNA, 2010, 2019a, 2019b). They consist of MM by settle plates, contact plates and glove prints in LAF/SC/I and settle plates in the background area. Recently, MM of the outer surface of ampoules and vials after disinfection by contact plates has been added to the standardised procedures. Media fills consist of process validation and the operator validation such as by the ‘Universal operator broth transfer validation’ (Boom and Beaney, 2015; UK Pharmaceutical Aseptic Services Committee, 2006). To evaluate aseptic handling in a broader context, the results of MM and media fills from over 40 Dutch hospital pharmacies are stored in Microbio, an Internet application for registration, evaluation and benchmarking of results of microbiological controls (Postma et al., 2012).

The working area (LAF/SC/I) can be considered as an EU Grade A environment and the recommended MM limits for this environment could be applied to aseptic handling too. At the moment these limits are < 1 cfu, calculated as an average value (EU GMP Annex 1, 2009). In the draft of the new Annex 1, the result in Grade A should be ‘no growth’ and if 1 or more cfu are found, this should result in an investigation (EU GMP Annex 1 revision, 2020). However, this new limit is not realistic for aseptic handling, because dragging of micro-organisms into LAF/SC by materials with a non-sterile surface cannot be avoided completely (Boom et al., 2019b). Moreover, aseptic handling is a...
manual activity inside LAF/SC, which makes contamination to some level inevitable (USP 35, 2012b). On the other hand, aseptic handling is executed with closed systems, which substantially reduces the risk of non-sterility comparing to open aseptic processing. Therefore, other limits for the working area than EU Grade A limits are justifiable.

A single MM sample is a snapshot in time and location (Denoya and Dalmaso, 2016). Therefore, aggregated results are necessary to get reliable information about microbial contamination of the aseptic processing environment and its control. Results need to be evaluated by trend analysis and compared with limits (Sandle and Vijayakumar, 2014; TR 13, 2014).

Because of the variability of microbial sampling methods and the limited accuracy of microbial growth, assessing of MM results on colony numbers is doubtful (Denoya and Dalmaso, 2016). Therefore, USP chapter <1116> advises assessing by an incident rate which is called the Contamination Recovery Rate (CRR). It is defined as the percentage of samples that show any microbial recovery, irrespective of the number of cfu (USP 35, 2012b). For example, an incident rate of 10% would mean that 10% of the samples taken have any contamination regardless of colony number.

Using the CRR is valuable, in particular, in circumstances with many samples with zero counts (Bar, 2015). These circumstances are found inside LAF/SC/I, which makes CRR a promising instrument for assessing MM data in aseptic handling.

The aims of this study are:

- to evaluate the different kinds of MM used inside, or related to, working in LAF/SC;
- to give background information about the MM limits used in Dutch hospital pharmacies;
- to discuss methods used for evaluating and assessing MM results.

In a subsequent article the MM results of approximately 40 Dutch hospital pharmacies from the previous 6 years will be discussed.

There is little experience with isolators in the Netherlands. Therefore, this study is restricted to aseptic handling performed in a LAF or SC.

2. Materials and methods

For this study, MM samples from air, gloves, worktop and starting materials with a non-sterile surface (ampoules and vials) from inside, or related to, working in LAF/SC were used. The sampling methods described below are a condensed version of the standardised procedures of Dutch hospital pharmacies (LNA, 2019a,b).

Definitions of terms, which are less common, are given in Appendix 1.

2.1. Air sampling using settle plates

- Sampling frequency: Every working day one settle plate (Tryptone Soya Agar 90 mm diameter, Biotrading Benelux) was used during one preparation.
- Sampling location: Near to the work zone*.

* Near to the work zone, but not in the work zone, because sampling itself should not pose a risk of contamination (EU GMP Annex 1, 2009).

- Sampling moment: Sampling started at the beginning of preparation, after the LAF/SC was filled with the components which were to be used during preparation.
- Sampling technique: The settle plate was opened and placed on top of its lid and was closed directly after preparation. Preparation times are short, which leads to short exposure times of around 15 – 30 min.

2.2. Glove print 5 fingers using settle plates

- Sampling frequency: Every working day one glove print of one of the operator's hands by a contact plate (Tryptone Soya Agar 90 mm diameter, Biotrading Benelux) was made.
- Sampling moment: After preparation and before glove disinfection.
- Sampling technique: The underside of the distal phalanxes of the thumb was pressed for at least 3 s on the agar surface and then the same for the 1st phalanxes of the other digits.

2.3. Worktop surface using contact plates

- Sampling frequency: Every working day one contact plate (Tryptone Soya Agar* 55 mm diameter, Biotrading Benelux) was used.

* The disinfectants used were ethanol 70% or isopropyl alcohol 70%, both of which evaporate completely. Therefore, there was no need for a disinfectant neutraliser in the agar.

- Sampling location: The work zone on the worktop of LAF/SC.
- Sampling moment: After preparation and before surface disinfection.
- Sampling technique: The contact plate was placed by a rolling movement* on the surface, the plate was pressed on the surface for at least 3 s and then removed by a rolling move.

* To prevent entrapment of air.

- After sampling, the agar residues on the worktop were removed by wiping with an alcohol impregnated wipe.

2.4. Outer surface of materials with a non-sterile surface using contact plates

The use of 10 samples of one kind of material (ampoule, vial) is recommended (Boom et al., 2019a). Disinfection took place in the background area. Operators wore appropriate clean room clothing, face masks and sterile gloves.

- One operator disinfected 10 ampoules or vials according to the local disinfection procedure and placed them into the LAF/SC.
- Sampling technique: An ampoule or vial was taken in the dominant hand and the contact plate in the other hand. The ampoule or vial was rolled slowly, with light pressure, from left to right and back afterwards (each for around 3 s) over the surface of a contact plate (Tryptone Soya Agar 55 mm diameter, Biotrading Benelux). The contact plate was turned and slow rolling was repeated twice. Care was taken not to touch the agar surface with the operators gloved fingers. For more information see reference Boom et al. (2019a).

* The disinfectants used were ethanol 70% or isopropyl alcohol 70%, both of which evaporate completely. Therefore, there was no need for a disinfectant neutraliser in the agar.

- After sampling, the agar residues on the ampoules and vials were removed by wiping with an alcohol impregnated wipe.

2.5. Incubation and cfu counting

- After sampling the agar plates were incubated for 7 days at 30 +/- 1 °C.
- Cfu were counted after 3 and 7 days.

2.6. Assessing and interpreting MM results

To assess MM results, mean cfu values and CRRs were used. For
trend analysis a ‘rolling CRR’ was developed, which calculates a CRR from a predetermined last number of samples for each sampling point. By using a spreadsheet template (Microsoft Excel 2016), the rolling CRR can be visualised in a diagram. The spreadsheet template is given in Appendix 2.

2.7. Statistics

The 95% confidence interval (CI) of the CRRs was calculated by using a method described by Newcombe (Newcombe, 1998). A calculator is available online (VassarStats, 2020). CRRs were compared by p-values using Fisher's exact test. For calculation of p-values, an online calculator was used (GraphPad QuickCalcs, 2020).

3. Results

3.1. MM results from hospital pharmacies

The 2018 MM results from 4 hospital pharmacies, expressed as mean cfu count and CRR, are summarised in Table 1. All mean cfu counts are below the level of 1 cfu. For mean cfu, as well as growth or no growth (expressed as CRR), the results for glove prints are the highest.

In Table 2, the 2018 CRRs for glove prints from the 4 hospital pharmacies of Table 1, are compared by Fisher’s test (GraphPad QuickCalcs). The results show that gloves from Hospital pharmacy 1 are significantly more contaminated compared to gloves from the 3 other hospital pharmacies.

In Table 3, the CRRs for glove prints within each hospital pharmacy over 4 years are compared using Fisher’s exact test (GraphPad QuickCalcs). The results from Hospital pharmacy 2 and 3 improved over time (due to better disinfection of materials with a non-sterile surface and raising the frequency of glove and worktop disinfection). Compared with 2015, these improvements were significant for Hospital pharmacy 2 in 2017 and 2018 and for Hospital pharmacy 3 in 2018. The low CRR of Hospital pharmacy 4 in 2016 is temporary.

In Table 4, the MM results for the surfaces of plastic and glass ampoules and injection vials are summarised. The number of samples of each kind of material are too low to calculate reliable values for CRRs (see subsection 4.5, Assessing MM results).

The results for glove prints of the non-dominant and dominant hand, or the left and right hand, from 4 hospital pharmacies are summarised in Table 5. If available, the results are divided into aseptic handling of non-hazardous products, and of antineoplastics. In all examples, the CRR of the non-dominant or left hand (around 90% non-dominant) is the highest.

3.2. Further results

In Fig. 2, the upper and lower limits of the 95% confidence interval (CI) for a CRR of 10% are expressed against sample size. Figs. 3 and 4 are examples of rolling CRR diagrams of glove prints using the last 100 samples. When new results are added, the CRR value from the last 100 samples is recalculated and the diagram is updated. Fig. 5 is the rolling CRR diagram of Fig. 3, in which a rolling CRR diagram, using the last 50 samples, is added.

4. Discussion

4.1. Sampling methods focused on aseptic handling

Viable air sampling can be divided into active air sampling by a volumetric air sampler and passive air sampling using settle plates. Active air sampling is general practice in the pharmaceutical industry for EU Grade A and B environments. However, during aseptic handling this is not advised, because the probe of the air sampler within the work zone in a LAF/SC is an additional risk of contamination and measuring outside the work zone will not reflect the situation within this zone. This point is also applicable for settle plates.

Additionally, due to the low percentage of samples with one or more cfu, confidence intervals are wide for sample sizes considered here (Petrie and Sabin, 2019). This makes meeting the required contamination level for air inside a LAF/SC by viable air sampling very difficult to achieve. Finally, the risk of air as a source of non-sterility is low because of working with closed systems during aseptic handling (Boom et al., 2020a; Doorne van et al., 1994; Stucki et al., 2009; Thomas et al., 2005). Therefore, based on the principles of risk assessment, the value of air sampling inside LAF/SC during aseptic handling is doubtful.

For monitoring of flat surfaces 55 mm diameter agar contact plates are recommended (Beaney, 2016). Swabs are advised for non-flat surfaces (Beaney, 2016). The recovery of contact plates and swabs is around 50% and 10% respectively (Beaney, 2016; Gouverde et al., 2017). In contrast to contact plates, swabs need additional laboratory handling before samples can be incubated. Therefore, even if the surface is not completely flat, it is advisable to use contact plates for surface monitoring (Boom et al., 2019a).

The longer the contact time between an object and the agar surface, the better the transfer of micro-organisms (Toschino et al., 2003). The 3 s mentioned in Section 2, Materials and Method, are a compromise
between theory and practice (LNA, 2019a). Enough contact pressure is also important for the transfer of micro-organisms. “The weight of a single finger resting on the plate while the seconds are counted” is a practical procedure (Beaney, 2016).

A previous study showed that touch of critical spots by gloved hands is the greatest risk of non-sterility (Boom et al., 2020a). Therefore, keeping the surface bioburden of these gloves as low as possible, as well as monitoring this surface, is extremely important (Boom et al., 2020b). This makes glove prints the most informative among all MM methods.

In Dutch hospital pharmacies, glove prints are made from the fingers of one hand only (Z3. Aseptic Handling, 2013). In the past, the dominant hand was advised, but there are good arguments to consider cfu counts on the non-dominant hand as more critical (for example, where a syringe is held in the dominant hand, the tip of the syringe or needle can be touched by the non-dominant hand). Additionally, the non-dominant hand holds disinfected ampoules and vials (surface can be contaminated, see Table 4) and the dominant hand holds syringes (sterile surface). Therefore, more cfu (a higher CRR) can be expected on the non-dominant, also the side where most cfu can be expected (see Table 5). Sampling the digits of both hands means more work and higher costs, but does not give additional information about keeping the contamination level of gloves as low as possible.

Materials with non-sterile surfaces, like ampoules and vials, can drag micro-organisms into LAF/SC, even after disinfection (Boom et al., 2019b). If these micro-organisms contaminate the hands of the operator, there is a risk of non-sterility (Boom et al., 2020a,b). Thorough disinfection of ampoules and vials can reduce this risk (Boom et al., 2019a). Therefore, the way in which this disinfection process has been carried out by operators must be verified. This is the reason why surface monitoring of ampoules and vials has recently been added to the Dutch MM procedures for aseptic handling (LNA, 2019a). However, taking samples of ampoules and vials during aseptic handling disturbs the preparation activities, and therefore is not advised (EU GMP Annex 1, 2009). Furthermore, glove prints indirectly reflect the surface bioburden of materials. This makes daily monitoring of disinfected ampoules and vials less of a necessity and allows for periodic monitoring (see subsection 4.2, Frequency of MM).

### 4.2. Frequency of MM

As mentioned in Section 2, Materials and Method, the advised frequency of MM for air, glove and worktop is once per working day. If a LAF or a SC is used daily, this will give around 250 samples each year for every kind of monitoring. If the aggregated results of MM are required rapidly, for instance if aseptic handling starts in a new facility, the MM frequency should be increased, initially to every work session. If these micro-organisms contaminate the hands of the operator, there is a risk of non-sterility (Boom et al., 2020a,b). Thorough disinfection of ampoules and vials can reduce this risk (Boom et al., 2019b). Therefore, the way in which this disinfection process has been carried out by operators must be verified. This is the reason why surface monitoring of ampoules and vials has recently been added to the Dutch MM procedures for aseptic handling (LNA, 2019a). However, taking samples of ampoules and vials during aseptic handling disturbs the preparation activities, and therefore is not advised (EU GMP Annex 1, 2009). Furthermore, glove prints indirectly reflect the surface bioburden of materials. This makes daily monitoring of disinfected ampoules and vials less of a necessity and allows for periodic monitoring (see subsection 4.2, Frequency of MM).

### Table 3

| Hospital | n | pos | neg | CRR (%) | n | pos | neg | CRR (%) | p1 |
|----------|---|-----|-----|---------|---|-----|-----|---------|----|
| Hospital 1 | 152 | 27 | 125 | 17.76 | 107 | 16 | 91 | 14.95 | 0.6130 |
| Hospital 2 | 188 | 34 | 154 | 18.09 | 290 | 39 | 251 | 13.45 | 0.1932 |
| Hospital 3 | 492 | 58 | 434 | 11.79 | 501 | 64 | 437 | 12.77 | 0.6991 |
| Hospital 4 | 420 | 24 | 396 | 5.71 | 294 | 5 | 289 | 1.7 | 0.0068 |

n = number of samples examined; pos = number of samples with one or more cfu; neg = number of samples without growth; CRR = Contamination Recovery Rate; p1 = p-value CRR 2016 compared to CRR 2015; p2 = p-value CRR 2017 compared to 2015; p3 = p-value CRR 2018 compared to CRR 2015.

### Table 4

| Hospital | pl amp | gl amp | inj | pl amp | gl amp | inj | pl amp | gl amp | inj |
|----------|--------|--------|-----|--------|--------|-----|--------|--------|-----|
| Hospital 3 | 20 | 30 | 20 | 90 | 50 | 30 | 40 | 30 | 30 |
| Hospital 4 | 3 | 2 | 1 | 5 | 0 | 0 | 1 | 6 | 3 |
| Hospital 5 | 17 | 28 | 19 | 85 | 50 | 30 | 39 | 24 | 27 |
| mean | 0.35 | 0.07 | 0.05 | 0.06 | 0.00 | 0.00 | 0.03 | 0.20 | 0.13 |
| CRR (%) | 15.00 | 6.67 | 5.00 | 5.56 | 0.00 | 0.00 | 2.50 | 20.00 | 10.00 |

n = number of samples examined; positive = number of samples with one or more cfu; negative = number of samples without growth; mean = mean cfu in samples examined; CRR = Contamination Recovery Rate.
consecutive samples, a higher sampling frequency (more than twice a day), to achieve the required 100 samples earlier, is likely to be inefficient and is therefore advised against. If, after 100 samples, the CRR is below the action limit, the frequency of sampling can be reduced to the standard frequency of once per working day. Limits are discussed later, in detail, in the subsection 4.4., Limits for CRR during aseptic handling.

To verify each operator's disinfection technique for materials with a non-sterile surface (ampoules and vials), it is advised that the MM of the outer surface of disinfected materials is added to the yearly audit of

Table 5
MM results for glove prints from the non-dominant and dominant hand, or from the left and right hand, from 4 hospital pharmacies.

|                  | Aseptic          | antineoplastic | Total        |
|------------------|------------------|----------------|--------------|
|                  | non-dom | dom | p1 | non-dom | dom | p2 | non-dom | dom | p3 |
| n                 | 274     | 273 | –  | 164     | 163 | –  | 438     | 436 | –  |
| positive          | 21      | 15  | –  | 18      | 13  | –  | 39      | 28  | –  |
| negative          | 253     | 258 | –  | 146     | 150 | –  | 399     | 408 | –  |
| CRR (%)           | 7.66    | 5.49| 0.3889 | 10.98    | 7.98 | 0.4507 | 8.90   | 6.42 | 0.2034 |

|                  | Aseptic          | antineoplastic | Total       |
|------------------|------------------|----------------|-------------|
|                  | non-dom | dom | p1 | non-dom | dom | p2 | non-dom | dom | p3 |
| N                 | 162     | 158 | –  | 151     | 153 | –  | 313     | 311 | –  |
| positive          | 18      | 7   | –  | 15      | 10  | –  | 33      | 17  | –  |
| negative          | 144     | 151 | –  | 136     | 143 | –  | 280     | 294 | –  |
| CRR (%)           | 11.11   | 4.43| 0.0358 | 9.93     | 6.54 | 0.3037 | 10.54  | 5.47 | 0.0262 |

|                  | Aseptic          | antineoplastic | total |
|------------------|------------------|----------------|-------|
|                  | left  | right | p1 | left  | right | p2 | left  | right | p3 |
| N                 | 851    | 851   | –  | 1332  | 1332  | –  | 2183  | 2183  | –  |
| positive          | 106    | 89    | –  | 160   | 121   | –  | 266   | 210   | –  |
| negative          | 745    | 762   | –  | 1172  | 1211  | –  | 1917  | 1973  | –  |
| CRR (%)           | 12.46  | 10.46 | 0.0164 | 12.01 | 9.08 | 0.0164 | 12.19 | 9.62 | 0.0075 |

Table 6
MM results from 2014 up to and including 2018 from a SC in Hospital pharmacy 2 and a LAF in Hospital pharmacy 4.

|                  | Hospital 2 SC | Hospital 4 LAF |
|------------------|---------------|---------------|
|                  | 2014 | 2015 | 2016 | 2017 | 2018 | 2014 | 2015 | 2016 | 2017 | 2018 |
| air n            | 280  | 199  | 290  | 219  | 298  | 418  | 412  | 334  | 298  | 247  |
| positive         | 6    | 6    | 4    | 7    | 10   | 10   | 9    | 0    | 1    | 2    |
| CRR (%)          | 2.14 | 3.02 | 1.38 | 10   | 10   | 2.39 | 2.18 | 0    | 0.34 | 0.81 |
| glove n          | 253  | 188  | 290  | 226  | 299  | 417  | 420  | 294  | 298  | 246  |
| positive         | 55   | 34   | 39   | 23   | 21   | 24   | 24   | 5    | 13   | 8    |
| CRR (%)          | 21.74| 18.09| 13.45| 10.18| 7.02 | 5.76 | 5.71 | 1.7  | 4.36 | 3.25 |
| worktop n        | -    | -    | 51   | 110  |      | 403  | 421  | 301  | 298  | 247  |
| positive         | 3    | 7    |      | 14   | 12   | 3    | 3    | 0    | 0    | 0    |
| CRR (%)          | 5.88 | 6.36 |      | 3.47 | 1.43 | 3.99 | 1.01 | 0    | 0    | 0    |

air: air sampling inside LAF/SC by settle plates; glove: glove prints of the operator; worktop: worktop surface inside LAF/SC; n = number of samples examined; positive = number of samples with one or more cfu; CRR = Contamination Recovery Rate.

aseptic = aseptic handling of non-hazardous products; antineoplastic = aseptic handling of antineoplastics; total = results of aseptic + antineoplastic; non dom = non-dominant hand; dom = dominant hand; left = left hand; right = right hand; n = number of samples examined; positive = number of samples with one or more cfu; negative = number of samples without growth; CRR = Contamination Recovery Rate; p1 = p-value CRR aseptic non-dominant/lefthand compared to CRR dominant/right hand; p2 = p-value CRR antineoplastic non-dominant/lefthand compared to CRR dominant/right hand; p3 = p-value CRR total non-dominant/lefthand compared to CRR dominant/right hand.
each operator (Boom et al., 2020b). If, during that audit, each operator disinfects 10 samples of one kind of material and 10 operators are involved with aseptic handling, 100 samples are monitored. These 100 will give a reliable prediction of the CRR of the outer surface of that kind of material after disinfection (see subsection 4.5, Assessing MM results). The following year the disinfection of a different kind of material can be tested in the same way.

4.3. Colony numbers versus growth or no growth

In Table 1, the MM results of LAF/SC for 4 hospital pharmacies are expressed as colony numbers (given as mean cfu) and as growth or no growth (given as CRR). In the introduction it has already been mentioned that assessing MM results based on colony numbers is of doubtful value because sampling cannot be standardised, the origin of one cfu can be one cell or a cluster of cells and there is large variability in microbiological assay recovery (USP 35, 2012b; Denoya and Dalmaso, 2016). Additionally, careful evaluation of MM results for colony numbers is difficult because the results do not fit standard statistical models, like a Poisson or a Normal distribution, including models allowing for overdispersion (Bar, 2015). These microbiological, as well as statistical, concerns are the reason that it is preferable to use CRRs instead of colony numbers to access MM results gathered in, or in connection with, a LAF/SC.

4.4. Limits for CRR during aseptic handling

Which CRR limit must be taken into account to demonstrate that aseptic handling is performed under adequate microbial control? For ISO 5 (= EU Grade A) the USP suggests a CRR < 1% (USP 35, 2012b). The results in Tables 1, 2 and 3 show that this value is difficult to achieve, even for passive air sampling.

After applying the precautions according to the Dutch standards for hospital pharmacies, thorough disinfection of materials and regular glove disinfection in particular, a CRR of less than 10% can easily be achieved (Z3. Aseptic Handling, 2013). The results from Hospital pharmacies 2 and 3 in 2018, as well as the results of Hospital pharmacy 4 from the whole 4 years period have demonstrated this (Tables 1 and 3).

A CRR of < 10% as well as a mean cfu count of < 1 cfu are used as limits in the MM procedures for aseptic handling in the Netherlands (LNA, 2019b). Both should be considered as an action level. Exceeding this level requires an investigation, and corrective actions based on the results of that investigation (TR 13, 2014). As is shown in Table 1, the CRR limit is more critical compared to the cfu limit.

The results in Table 4 show that it is difficult to achieve a low surface bioburden of plastic and glass ampoules and injection vials after disinfection. This was also confirmed in a previous study (Boom et al., 2019b). However, until more results are available, a CRR limit of less than 10% for MM results of disinfected materials is also advised.

4.5. Assessing MM results

Fig. 2 illustrates that the reliability of a CRR depends on sample size. Therefore, to robustly report a particular CRR, always describing a CRR together with the number of samples is recommended. For example, a CRR_{100} is calculated using 100 samples and a CRR_{250} is calculated using 250 samples.

Not only this study (Table 1), but also results in Microbio, illustrate that during aseptic handling MM results for gloves are the highest (Postma et al., 2012). Therefore, the following section, mainly focuses on the results for glove prints.
Tables 2 and 3 illustrate a large number of samples and/or a great difference in CRR is necessary to demonstrate a statistically significant deviation above a limit at the customary alpha level of 5%. This is illustrated in Fig. 2, which shows large 95% CIs at small sample sizes. These large numbers make statistical significance (at an alpha level of 5%) a less suitable instrument for determining upward or downward trends of MM results.

Barr showed a method for assessing MM results using CRR, in which the results per unit of time (for example a month) or per fixed number of samples (for example 100) are compared with cumulative results from a longer period (Barr, 2015). Because of the changing number of samples per unit of time, a CRR calculated using a fixed number of samples is preferred. For cumulative results the mean CRR of the last year or a longer period was used (for a robust value at least 250 samples is advised, see Fig. 2). These cumulative results are called the ‘reference value’ and concern a particular sampling method and sampling point. For example, the reference values for air sampling and glove prints of Hospital pharmacy 2 are 3% and 7% respectively and for Hospital pharmacy 4 these values are 1% and 4% respectively (see Table 6).

For assessing MM results by trend analysis, the rolling CRR has been developed (see Section 2, Materials and Methods). If this is calculated using the last 100 samples, the diagram is called a ‘rolling CRR$_{100}$ diagram’ (see Figs. 3 and 4). If the number of samples is less, the frequency, as well as the magnitude of the upward and downward changes, will increase. This is shown in Fig. 5, in which a rolling CRR$_{50}$ diagram is added to the rolling CRR$_{100}$ diagram of Fig. 3. Choosing a lower number of samples leads to increased fluctuations and allows detection of upward trends earlier but at the cost of more false positives. Therefore, the choice of the number of samples to be used involves a trade-off between timely detection and reliability of the rolling CRR value. A rolling CRR$_{100}$ is a good compromise between both.

Figs. 3 and 4 are the rolling CRR$_{100}$ diagrams for glove prints from 2019 of a SC of Hospital pharmacy 2 and of a LAF of Hospital pharmacy 4. As mentioned above, the reference values are 7% and 4% respectively. If the results of 2019 are in accordance with these values, the rolling CRR$_{100}$ diagram will move upwards and downwards compared to 7% and 4% respectively. Based on practical considerations, an upward trend of the rolling CRR$_{100}$ is defined as an increase above the reference value by at least 2% (percentage point) during a period of at least one month. This threshold defines the alert level. This happened at the end of 2019 in Hospital pharmacy 4 (rolling CRR$_{100}$ > 6%, see Fig. 4). In Hospital pharmacy 2, this happened twice for a longer period in the second part of 2019 (rolling CRR$_{100}$ > 9%, see Fig. 3). The duration, as well as the increase of the rolling CRR$_{100}$ up to 14%,
indicates that the process is not in control.

If the reference value is low, the risk of a sample with one or more cfu is obviously also low. To prevent unnecessary alerts, fixing the minimum reference value at 4% is recommended, which makes the minimum alert level > 6%. Most reference values for glove prints are above 4% (see Table 3). By contrast, reference values of settle plates and worktop prints are in general below 4% (see Tables 1 and 6).

4.6. Documentation of MM results

Results of periodical data analysis, as well as MM data from longer periods should be documented (TR 13, 2014). An example of data analysis is described in Appendix 3. Fig. 1 and Table 6 are examples of documentation. Together, they give a good overview of MM results during a longer time period for a particular LAF or SC. After adding new results in Table 6, reference values can be recalculated. The number of samples on the worktop surface in the SC of Hospital pharmacy 2 are too small for calculating a reliable reference value (see Table 6).

4.7. Limitations

Some limitations of this study are as follows:

A single MM sample is a snapshot in time and location. The smaller the percentage of samples with growth and the lower the number of samples taken, the lower the reliability of the calculated CRRs. As the number of samples taken cannot be increased unlimitedly, this puts an upper limit on the accuracy with which CRRs can be assessed. These restrictive statistical possibilities also have consequences for detecting upward or downward trends, based on MM results, and may lead to time lags until such trends can be clearly proven.

5. Conclusion

Of all MM methods, glove prints are the most informative. The added value of air sampling is doubtful. Because of microbiological, as well as statistical, considerations using CRR for assessing MM results is advised. Glove prints, in general, give the highest CRR. A CRR < 10% is a realistic limit for MM during aseptic handling in hospital pharmacies. A rolling CRR using the last 100 samples is a good compromise between reliability of the CRR value and timely predicting of process changes.

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Credit author statement

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2020.105540,

Appendix A

Definitions

Appendix B

Excel template for drawing a rolling CRR100 diagram

Appendix C

Data analysis by reference values and rolling CRR100

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