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# ESTABLISHMENT OF METHOD FOR BIOBURDEN RECOVERY: NON-ANTIBIOTIC ORAL TABLETS (STUDY II)

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## ABSTRACT

Determination of the microbiological quality of the final pharmaceutical products is crucial not only to assess the degree of the compliance of the manufacturing process in the pharmaceutical facility to Good Manufacturing Practice (GMP) but also to the safety of the drug to the patients' consumption. If the applied microbiological testing method is not examined for its suitability for microbial enumeration and detection for each product situation a false estimation of bioburden may occur which encounter great risk for both consumer and manufacturer. A risk assessment study using tabular method was performed primarily to identify potential hazard associated with microbial contamination of oral tablets. A survey study for a group of 15 drugs of different non-sterile, non-antibiotic oral tablets from the market were tested for Method suitability (MS) using selected standard strains of bacteria and fungi after ensuring the purity of cultures by miniaturized identification biochemical system (BBL Crystal and API20C AUX) and characteristic morphological appearance. Growth media used in the study are those that passed growth promotion tests. The primary applied technique is the dilution as a mean of sample processing and neutralization of any non-antibiotic antimicrobial activity. Then chemical neutralization is followed if dilution alone could not recover one or more microbes effectively. Dilution technique proved to be adequately effective at 1:10, 1:20, 1:40 and 1:100 (w/v) with the later dilution being the most common for microbial enumeration and the first dilution for specific microorganism detection. No chemical neutralization was needed during the course of the study.

**KEYWORDS:** Good manufacturing practice, Microbial enumeration and Detection, Non-antibiotic oral tablets, Bioburden, Method suitability, Standard strains.

## INTRODUCTION

Microbial contamination costs companies thousands to millions of dollars annually through the machines damage, production downtime, product contamination, investigations, and energy losses. The scope of the majority of reputable companies; nowadays, is focused on understanding the sources of contaminants.[1]

Pharmaceutical products are subject to microbiological contamination that can represent a health hazard to the consumer and cause product spoilage, aesthetic changes, and possible loss of drug efficacy. Microbial contamination may originate from the raw materials and excipients or may be introduced during manufacture (operators and contaminated equipment, environment, and packaging materials), storage and use. Most raw materials used in pharmaceutical manufacturing, including water, may contain several types of microorganisms. Depending on the type of the manufacturing process, these contaminants may be reduced or eliminated. However, care must be taken not to further increase the potential for introducing microorganisms during an uncontrolled manufacturing process.[2]

The principle of risk assessment as a tool to improve pharmaceutical processes was introduced by the FDA.[3] Process risk assessment tool have been successfully used by pharmaceutical companies to identify areas in the process and types of raw materials and equipment that are at high risk of being contaminated with microorganisms.[4]

The current study aimed to perform risk assessment of microbiological contamination for oral non-antibiotic tablets then applying it through method validation on selected group of film coated and uncoated tablets from the market to elucidate the ability of the method to recover certain microbial species and to modify the method as necessary to fit each formulation.

## MATERIALS AND METHODS

A tabular risk assessment as described by Sandle was applied to oral tablets.[5] Through risk analysis critical functions are identified and validation studies are focused on certain aspects. Method suitability study was conducted on selected tablet products as listed in Table (1). Standard strains were handled and prepared as standard method of ATCC from which they were purchased. All media of the study were tested for growth promotion as described in compendial methods. Identification and purity check of microorganisms were done as stated by some authors.[6,7] Test for specified microorganisms, for microbial enumeration and

growth promotion of culture media and changes in standard microbiological method was done according to Clontz, 2008 for method validation with modification.[2]

A modification of the ordinary method of sample preparation and dilution was done in one or more of the following cases

1- Inhibition of the microbial recovery either in the enumeration or detection techniques.
2- Interference from product in microbial counting and detection methods such as highly turbid products that forms very opaque background which affects the process of counting.
3- Pharmaceutical preparation yield unacceptable product for further processing e.g. firm gel that cannot be withdrawn.
4- Required amount of tablets are not feasible for economic reasons such as small amount of batch size (R and D, trial or free medical sample batches) in addition to high cost of pharmaceutical preparations and small weight of dosage forms.

**Table (1): List of non-antibiotic oral film coated and uncoated tablets subjected to neutralization method validation showing active pharmaceutical ingradients (API), other ingredients and their medicinal use.**

| Code Name | API | Concentration | Excipient |
|---|---|---|---|
| ATL | Sennoside Calcium 60% | 12.5 mg/tab. | Avicel PH 101, Talc, Lactose [(a)] and Magnesium Stearate. |
| PLC | Leflunomide | 20 mg/tab. | Crospovidone, Povidone, Lactose [(a)], Magnesium Stearate and Aerosil 200. |
| HAM | Cilostazol | 100 mg/tab. | Sodium Croscarmellose, HPMC, Magnesium Stearate, Avicel PH 102 and Maize Starch. |
| LAS | Ebastine | 10 mg/tab. | Lactose [(a)], Avicel PH 102, Sodium Croscarmellose, Magnesium Stearate, Aerosil 200 and Snowflaks. |
| AMP | Itopride Hydrochloride | 50 mg/tab. | Lactose, Aerosil 200, Avicel PH 101, Maize Starch, Magnesium Stearate and PVP. |
| LEA | Calcium Rosuvastatin | 10.4 mg/tab. | Lactose [(a)], Emcompress [(c)], Crospovidone, Avicel PH 102, Magnesium Stearate and Opadry. |
| PCC | Sodium Montelukast | 10.92 mg/tab. | Avicel PH 101 and 102, Sodium Croscarmellose, Aerosil 200, Magnesium Stearate, Opadry and Purified Water USP. |
| CAC | Paracetamol, Chlorpheniramine Maleate, Pseudoephedrine Hydrochloride | 500, 2, 30 mg/tab. | Avicel PH 101, Sodium Croscarmellose, PVP, Aerosil 200, Magnesium Stearate, PEG, Titanium Dioxide, Sunset Yellow [(b)] and Purified Water USP. |
| TOL | Valsartan, Amlodipine Besylate | 160, 6.9 mg/tab. | Crospovidon, Avicel PH 102, Aerosil 200, Sodium Starch Glycolate, Magnesium Stearate, |

| | | | |
|---|---|---|---|
| | | | HPMC, Talc, Titanium Dioxide, PEG, Iron Oxide Yellow and Purified Water USP. |
| ALA | Diosmin, Hisperidine | 450, 50 mg/tab. | Avicel PH 101, Lactose [(a)], Sodium Croscarmellose, Aerosil 200, PVP, Magnesium Stearate, HPMC, Titanium Dioxide, FD and C Yellow no.10, Ponaceau 4R and Purified Water USP. |
| LMT | Chlordiazepoxide, Clidinium Bromide | 5.25, 2.625 mg/tab. | Lactose [(a)], Maize Starch, Talc, Magnesium Stearate, HPMC, Opadry and Ethanol. |
| EAA | Bisoprolol Fumarate, Hydrochlorothiazide | 5, 12.5 mg/tab. | Maize Starch, Emcompress [(c)], Aerosil 200, Magnesium Stearate, Avicel PH 101, HPMC, PEG, Titanium Dioxide, Talc, Red and Yellow Iron Oxide and Purified Water USP. |
| AMA | Fexofenadine Hydrochloride | 120 mg/tab. | Sodium Croscarmellose, Avicel PH 101 and 102, Maize Starch, Magnesium Stearate, Ethanol 96%, Aerosil 200, HPMC, PEG, Titanium Dioxide and Purified Water USP. |
| SPM | Glimepiride | 2 mg/tab. | Lactose [(a)], Sodium Starch Glycolate, PVP, Magnesium Stearate, Avicel PH 102 and Opadry. |
| ACA | Minerals, vitamins and trace elements | NA | Avicel, Crospovidone, Aerosil 200, HPMC, Opadry and Ethanol. |

NA= Not applicable due to unavailability of data from the manufacturer. Aerosil 200= Hydrophilic Fumed Silica and the number indicates specific surface area in $m^2/g$.

PVP= Polyvinylpyrolidone (Povidone). PEG= Polyethelyne Glycol 4000 and 6000.

Avicel= Microcrystalline Cellulose. HPMC= Hydroxypropylmethylcellulose 3cps or 15cp.

(a) = Monohydrate form. (b)= FD and C Yellow no.6. (c)= Dibasic Calcium Phosphate, Dihydrate

All experimental testing were done in Biological safety Cabinet (BSC) (Jouan MSC 9 Class II A2 BioSafety Cabinet, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, California 95134). All the nutrient media and chemicals were purchased from OXOID (Basingstoke, Hampshire) and Sigma-Alrich (St. Louis, MO 63103), respectively. All organisms were stored at -80°C in a validated -86C Ultra low temperature freezer (-86 Degree ULT Freezers, Qingdao Shandong, China) in a validated cryogenic environment, and revived prior to conducting the study. All culture media were sterilized by autoclaving in steam sterilizer (FEDEGARI FOB4L-TS, Fedegari Autoclavi SpA, SS 235 km 8, 27010 Albuzzano (PV), Italy). Microbial test suspensions were used once the results of serial dilutions could be enumerated using digital colony counter (Digital Colony Counter Model: 361, Laxman Mahtre Rd. Navagaon, Dahisar West, Mumbai). All statistical analysis was

performed using GraphPad Prism® v6.01 for Windows. Any interpretation or complex calculation was performed using Microsoft Excel 2007.

## RESULTS AND DISCUSSION

Risk assessment analysis was performed using tabular method as described in Table (2) showing the importance of the verification of the suitability of the method for bioburden analysis and identified its role and importance for finished pharmaceutical products as well as their raw materials from which they are manufactured. This should be isolated from other measures that should be taken to ensure safety and efficacy of drug product. Initial assessments of bioburden quality of the tested non-sterile pharmaceutical products –using conventional methods- revealed that the tested products were clean microbiologically. All standard strains were verified for their purity and identity.

Test for bioburden enumeration was successful as illustrated in Table (3) at dilutions specified in Table (4). Test for specified microorganisms was successful for all tested products at dilutions specified in Table (4). For microbial enumeration 1:100 (w/v) dilution ratio yielded satisfactory results with most of the products while for test of specified microorganisms 1:10 (w/v) ratio was the most applied dilution for most of the tablets. Negative control samples from used culture media did not show any microbial growth. Environmental monitoring samples taken during performing microbiological tests in the BSC did not show any microbial growth neither from air samples nor surface samples.

**Table (2): The Tabular Approach of risk assessment for the bioburden analysis of the non-antibiotic oral tablets.**

| **Affected Product: Non-antibiotic non-sterile oral tablets** | | |
|---|---|---|
| **Risk: Contamination due to build up of bioburden during pharmaceutical product manufacturing** | | |
| **Failure or Situation: Failure to adequately detect microbial contamination in the final finished product** | | |
| **Effect** | **Minimizing the risk (Mitigations to reduce risk)** | **Monitoring** |
| - If the bioburden detection procedure is not able to recover microbial contamination efficiently, there is a risk to patient health and wrong assessment of product quality which impacts the reputation of the manufacturing | - Microbiological analysis of incoming raw materials after applying proper method validation and risk analysis.<br>- Strict application of GMP through training, proper machine and heating, ventilating, and air conditioning (HVAC) system maintenance, cleaning validation and clean rooms sanitization.<br>- Screening of the products for both microbial enumeration and detection methods at the initialization phase of pharmaceutical dosage form | - For out of specification results (OOS) there should be a corrective and preventive action (CAPA) in place to act upon.<br>- Adjusting the testing frequency after reaching steady state and reviewing the microbial trend for each product.<br>- Environmental and personnel gown monitoring samples at |

| | | |
|---|---|---|
| facility.<br>- A falsely apparently microbiologically clean product is masking possible violations in good manufacturing practice (GMP) which would not be otherwise corrected. | launch.<br>- Applying modifications as necessary to suite each type of product in order to improve the ability of the method for microbial cells recovery.<br>- Reapplying method validation if significant change has been made to the pharmaceutical dosage form.<br>- Microorganisms that could not be recovered by any neutralization methods are of low risk hazard. | appropriate frequency depending on critical area and processes through production manufacturing facility.<br>- Proper monitoring with appropriate frequency of pharmaceutical water facility that is used for cleaning, sanitization and drug manufacturing. |

**Table (3): Method suitability enumeration survey study performed on selected oral tablet products showing the ratio of microbial recovery of each product relative to the positive control.**

| Product Code | Relative Microbial Recovery | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Bacillus subtilis*** | ***Pseudomonas aeruginosa*** | ***Candida albicans*** [(a)] | | ***Aspergillus niger*** [(a)] | |
| | 30-35°C | 30-35°C | 30-35°C | 20-25°C | 30-35°C | 20-25°C | 30-35°C |
| ATL | 1.17 | 1.02 | 1.10 | 0.62 | 0.89 | 0.52 | 0.63 |
| PLC | 0.78 | 1.06 | 1.23 | 0.75 | 1.24 | 0.73 | 0.61 |
| HAM | 1.07 | 0.83 | 1.16 | 0.62 | 1.06 | 1.12 | 0.59 |
| LAS | 1.16 | 1.11 | 1.22 | 0.62 | 0.56 | 0.70 | 1.00 |
| AMP | 1.23 | 1.10 | 1.17 | 0.51 | 0.86 | 0.80 | 0.59 |
| LEA | 0.82 | 0.82 | 1.09 | 0.81 | 0.66 | 1.09 | 0.64 |
| PCC | 1.00 | 0.89 | 0.99 | 0.98 | 0.86 | 0.90 | 0.79 |
| CAC | 1.08 | 0.89 | 0.99 | 0.84 | 1.00 | 0.94 | 1.01 |
| TOL | 0.88 | 0.69 | 0.91 | 1.14 | 1.06 | 1.27 | 0.97 |
| ALA | 1.04 | 0.79 | 0.98 | 0.51 | 1.06 | 1.00 | 0.76 |
| LMT | 1.26 | 1.08 | 1.21 | 0.54 | 0.53 | 1.02 | 1.03 |
| EAA | 1.25 | 0.88 | 1.13 | 0.58 | 0.54 | 1.00 | 0.99 |
| AMA | 0.99 | 0.94 | 0.92 | 0.69 | 0.91 | 0.67 | 0.79 |
| SPM | 1.40 | 0.96 | 1.14 | 0.63 | 1.17 | 0.51 | 1.06 |
| ACA | 0.95 | 1.08 | 1.11 | 0.86 | 0.82 | 0.68 | 1.03 |

(a)= Dual temperature incubation at 20-25°C and 30-35°C on Sabouraud Dextrose Agar (SDA) and Tryptone Soya Agar (TSA) respectively.

No other techniques (principally filtration or chemical neutralization) for neutralization were necessary. Oral tablets were not different in terms of total microbial recovery from each other at specified dilutions as shown in Fig. (1). Interestingly, it was found that fungi recovery was significantly lower than that of bacteria especially *Candida albicans* from SDA at 20-25°C. Microbial recovery for bacteria was higher for *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* then *Bacillus subtilis*. This finding is demonstrated in Fig. (2).

**Table (4): Applied technique(s) in method suitability for each tested coated and uncoated tablets showing the inoculums level used of the test microorganisms for each material.**

| Raw Material | Inoculum Range* | Neutralization for Enumeration | | | Inoculum Range* | Neutralization for Detection of Specific Microorganisms | | |
|---|---|---|---|---|---|---|---|---|
| | | Dilution | Filtration | Chemical | | Dilution | Filtration | Chemical |
| ATL | 21-90 | ++++ | - | - | 50-98 | + | - | - |
| PLC | 21-88 | ++++ | - | - | 50-98 | ++++ | - | - |
| HAM | 15-88 | ++++ | - | - | 50-98 | + | - | - |
| LAS | 28-86 | ++++ | - | - | 50-98 | + | - | - |
| AMP | 21-95 | ++++ | - | - | 50-98 | + | - | - |
| LEA | 21-99 | ++++ | - | - | 50-98 | + | - | - |
| PCC | 24-99 | + | - | - | 50-98 | + | - | - |
| CAC | 28-94 | +++ | - | - | 30-68 | +++ | - | - |
| TOL | 15-80 | ++ | - | - | 50-98 | ++ | - | - |
| ALA | 23-86 | ++++ | - | - | 50-98 | + | - | - |
| LMT | 20-95 | ++++ | - | - | 50-98 | + | - | - |
| EAA | 23-90 | ++++ | - | - | 38-68 | + | - | - |
| AMA | 21-93 | ++++ | - | - | 50-98 | + | - | - |
| SPM | 12-96 | ++++ | - | - | 50-98 | + | - | - |
| ACA | 29-84 | ++++ | - | - | 50-98 | ++++ | - | - |

+= 1:10 (w/v). ++= 1:20 (w/v). +++= 1:40 (w/v). ++++= 1:100 (w/v). *= Mold count ranged from 8 to 80 CFU while bacteria and yeast from 30 to less than 100 CFU.

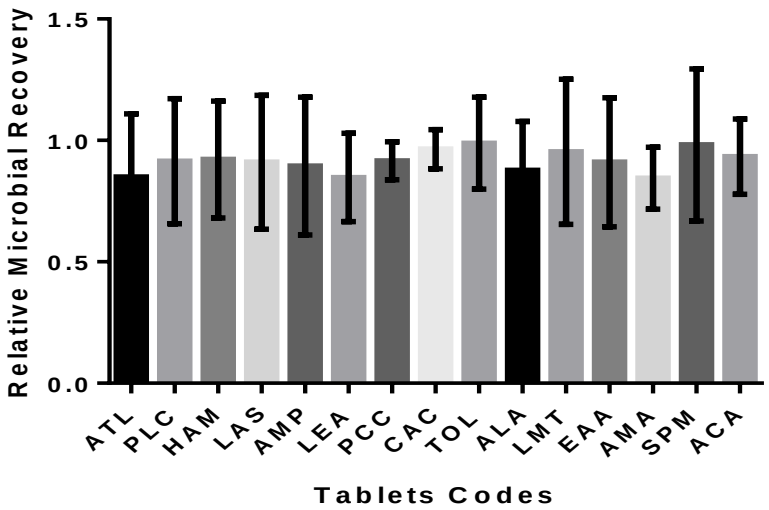
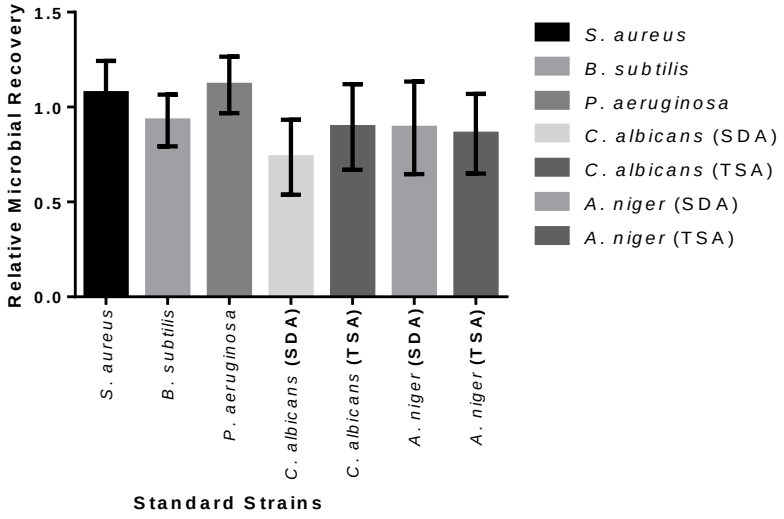



**Figure (1): Difference in total microbial recovery between tested 15 tablets showing average of the ratio of microbial recovery for each product ± S.D. (Data generated by GraphPad Prism® v6.01 for Windows)**




**SDA= 20-25°C. TSA= 30-35°C.**

**Figure (2): Difference in microbial recovery within-group of 15 tablets showing average of the ratio of microbial recovery for each microorganism ± S.D. (Data generated by GraphPad Prism® v6.01 for Windows).**

Bracketing technique is a helpful tool if there are products close in composition e.g. Ham, PCC and AMA are available in both (50, 100), (5, 10) and (120, 180) mg of API in the market with similar excipients. Many products can be thought of as existing in families, with minor variations in formulation or unit fill volume. One approach to the issue of having to perform method suitability testing on so many products is to try to put together a testing plan that considers these product family groups.[8]

The use of dual media and dual incubation temperature range for fungi is based on the fact that TSA can support the growth of both bacteria and fungi. Although the microbial limit tests call for the use of the Sabouraud Dextrose medium for the recovery of fungi, studies performed over the years have demonstrated that all-purpose media, such as Soybean Casein Digest (SCD), are capable of recovering a wide range of bacteria, yeasts, and molds.[9,10]

The current study showed the ability of the procedure to recover low level microbial contamination from the tested coated and uncoated tablets. However, it should be complemented with hold time study for time not exceeding 1 hour as discussed by Clontz 2008.[2] But this study could not be accomplished unless an effective recovery method has been proven.

## CONCLUSIONS

The method applied for microbial enumeration and detection using selected standard strains by dilution technique alone was effective for the currently used non antibiotic tablets. The risk assessment made for single category of pharmaceutical class of products such as oral solid dosage forms can be further broken into individual risk analysis for each product specifically.

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## REFERENCES

1. Eissa ME, Abd El Naby M, Beshir MM. Bacterial vs. fungal spore resistance to peroxygen biocide on inanimate surfaces. Bulletin of Faculty of Pharmacy, Cairo University, 2014; 52(2): 219-24.
2. Clontz L. Microbial limit and bioburden tests: validation approaches and global requirements. 2nd ed., New York; CRC Press: 2008.
3. FDA. Pharmaceutical cGMPs for the 21st Century—A Risk-Based Approach Final Report. Department of Health and Human Services, U.S. Food and Drug Administration, 2004.
4. WHO. Application of Hazard Analysis and Critical Control Point Methodology to Pharmaceuticals. In WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-Seventh report. Geneva. World Health Organization, 2003, Annex 7 (WHO Technical Report Series, No. 908).
5. The use of risk assessment tools for microbiological assessment of clean room environments, Compilation prepared by Tim Sandle, http://www.insightcgmp.com/gmp-guru/risk-assessment-tools.pdf.
6. Estridge BH, Walters NJ, Reynolds A. Basic Medical Laboratory Techniques. 4th ed., Delmar, USA; Thomson Learning: 2000.
7. Eissa ME. Studies Of Microbial Resistance Against Some Disinfectants: Microbial Distribution & Biocidal Resistance in Pharmaceutical Manufacturing Facility. 1st ed., Saarbrücken; LAP Lamber Academic Publishing: 2014.

8. Does International Harmonization of the USP Microbial Limits Tests Require Re-Validation of Finished Product Tests?, Coordinated by Scott Sutton. http://www.ivtnetwork.com/sites/default/files/InternationalHarmonization_01.pdf.
9. Marshall V, Poulson-Cook S, Moldenhaver J. Comparative Mold and Yeast Recovery Analysis (the effect of differing incubation temperature ranges and growth media). PDA J Pharm Sci Technol, 1998; 52(4): 165–9.
10. Cundell AM. Review of the Media Selection and Incubation Conditions for the Compendial Sterility and Microbial Limit Tests. Pharmacopeial Forum, 2002; 28(6).