*Available online at* www.scholarsresearchlibrary.com

Scholars Research Library

**Der Pharmacia Lettre, 2015, 7 (10):72-75**
***(http://scholarsresearchlibrary.com/archive.html)***

Der Pharmacia Lettre
Scholars Research Library
**ISSN 0975-5071**
**USA CODEN: DPLEB4**

# Microbiological examination of pharmaceutical raw materials

**Rajapandi. Senthilraj*, Ganduri Sathyanarayana Prasad and Kunchithapatham Janakiraman**

*Department of Pharmacy, Annamalai University, Annamalainagar, Chidambaram, India*

---

**ABSTRACT**

*In order to manufacture medicines of acceptable microbiological quality, it is necessary to know the microbial contaminants resulting from raw materials. The microbiological quality of the final product (except manufacturing process) depends only on the quality of the raw materials. Hence it was aimed to assess microbiological quality of fourteen raw materials by conventional methods. Among the chosen samples; Gelatin, Lactose, Sodium alginate and Xanthan gum had higher microbial count and the remaining samples had only few or negligible microbial count in Soyabean-Casein digest broth. Microbial growth was observed in selective media for the samples viz., Thyroxinesodium, Acacia, dried Aluminium hydroxide gel, Gelatin, Lactose, Liquid Paraffin, Sodium alginate, Starch and Xanthan gum. Niacinamide, Magnesium hydroxide, Paracetamol Riboflavin and Tragacanth had not shown any microbial growth on selective media. No selected sample was found to contain any objectionable microorganism. The conventional methods of identification required more than 7 days for completion, so new methods should be followed to avoid wastage of time. The above results indicate that raw materials must be assessed for their microbiological quality, in order to produce quality medicines.*

**Keywords:** Raw materials, Most probable method, Microbes in raw materials, Microbial quality, Quality of Raw materials.

---

## INTRODUCTION

Microbiological quality for raw materials is one of the necessary requirements to accomplish the 'Good Manufacturing Practices' in the Pharmaceutical Industry (1). Control of microbiological contamination of the raw materials is most important, because microorganisms may contaminate the finished product as well as the manufacturing plant, which might cause an intermittent or continuous pollution of the product that is very difficult to eliminate (2). Excipients are the most influential amongst the ingredients in this type of contamination of medicaments, since the microorganisms that appear in the raw materials can be the origin of diseases or may cause spoilage of the medicaments(3,4). The microbiological quality of the final product (except manufacturing process) depends only on the quality of the starting materials (5,6). Hence the present work was aimed to assess the microbiological quality of some raw materials, which are commonly used in Pharmaceutical industries for the manufacture of non-sterile pharmaceutical products, as well as to find out whether these raw materials confirm the microbiological specifications of the United States of Pharmacopoeia and Indian Pharmacopoeia.

## MATERIALS AND METHODS

A total number of the fourteen Raw materials were chosen for the study. They were: Acacia, Gelatin, Lactose, Starch, Sodium alginate, Tragacanth, Xanthan gum. Dried Aluminium hydroxide gel, Liquid paraffin, Magnesiumhydroxide, Niacinamide, Paracetamol, Riboflavin and Thyroxine sodium. Samples were obtainedfrom different Pharmaceutical Industries which bought them from various suppliers. Culture media and other chemicals were supplied by M/s Himedia, Mumbai.

**1. Microbiological examination of Raw materials:**

**1.1. Preparation of the sample:**

1:10 dilutions of all raw materials were made aseptically with soybean-casein digest broth and the same broth was used to make further dilution(1:100). Polysorbate 80(1gm/litre) was added to solubilise non-fatty water insoluble samples(1,7).

**1.2.Determination of Total Viable Count by Most Probable Number(MPN) method:**

From the 1:10 dilutions of raw materials(as mentioned above), serial dilutions of 1:100 and 1:1000 were prepared using the same broth(3 tubes for each dilution), to get concentrations of 0.1g or 0.1ml ,0.01g or 0.01ml and 0.001gm or 0.001ml of test samples respectively. The above sets of tubes were incubated with control (soyabean-casein broth) at 37°C for 48 hours (8,9,10).

**1.3. Tests for specified Micro-organisms:**

A 1:10 dilution of the raw materialin soybean casein digest broth was used for the isolation of all specified microorganism, after incubation at $35^0$Cfor 5 to24 hours.In order to increase the number of particular microorganism present in the raw materials, suitable enrichment broth were used. Enrichment brothsused were Enterobacteria enrichment broth mossel for enterobacteria, MacConkey broth for *Escherichia coli*, Rappaport Vassiliadis *Salmonella* enrichment media for *Salmonella*, GN media for *Shigella*sp, Reinforced medium for *Clostridia*, Sabouraud dextrose broth for *Candida albicans* respectively. Further the isolation was performed using the following agar media viz., , violet red bile glucose agar, MacConkey agar, Xylose lysine deoxycholate agar, Cetrimide agar, Mannitol salt agar, Columbia agar and sabouraud dextrose agar with respect to specified microorganism, Gram negative bile tolerant enterobacetria, *Escherichiacoli*,*Salmonella*sp, *Shigella*sp, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridia* sp. and *Candida albicans* (1,7,11,12).

## RESULTS AND DISCUSSION

**Table:1Number of viable aerobic bacteria in raw materials**

| S.No | Raw material | Most probable Number of microorganisms/ml [or]gm |
|---|---|---|
| I. | Acacia | 1100 |
| I. | Gelatin | >1100 |
| I. | Lactose | >1100 |
| V. | Sodium alginate | >1100 |
| V. | starch | 1100 |
| I. | Tragacanth | 3 |
| I. | Xanthan gum | >1100 |
| I. | Dried Aluminiumhydroxide gel | 7 |
| X. | Liquid paraffin | 11 |
| X. | Niacinamide | 3 |
| I. | Magnesium hydroxide | 4 |
| I. | Paracetamol | 3 |
| I. | Riboflavin | 3 |
| V. | Thyroxine sodium | 20 |

**Table: 2 Thegrowth pattern of microorganisms on selective media from Raw materials**

| S.no | Tested samples | VRG-Bg.neg | MAC-*E.coli* | XLD-Sal | XLD-Shig | CET-P.aue | MAN-S.aur | COL-Clost | SAB-C.alb |
|---|---|---|---|---|---|---|---|---|---|
| I. | Acacia | - | - | + | - | - | + | - | - |
| I. | Gelatin | - | + | - | - | - | + | - | - |
| I. | Lactose | - | - | - | - | - | + | - | - |
| /. | Sodium alginate | - | - | - | - | - | + | - | - |
| /. | starch | - | - | - | - | - | + | - | - |
| I. | Tragacanth | - | - | - | - | - | - | - | - |
| I. | Xanthan gum | - | - | - | - | - | + | - | + |
| I. | Dried aluminium. hydroxide gel | + | - | - | - | - | + | - | - |
| K. | Liquid paraffin | - | - | - | - | - | + | - | - |
| K. | Niacinamide | - | - | - | - | - | - | - | - |
| I. | Magnesium hydroxide | - | - | - | - | - | - | - | - |
| I. | Paracetamol | - | - | - | - | - | - | - | - |
| I. | Riboflavin | - | - | - | - | - | - | - | - |
| /. | Thyroxine sodium | + | + | + | + | - | + | - | - |

*VRG-Violet Red bile Glucose agar, MAC-MacConkey agar, XLD-Xylose Lysine Deoxycholateagar, CET-Cetrimide agar, MAN-Mannitol salt agar, COL-Columbia agar, SAB-Sabouraud Dextrose agar.*
*+ indicates growth and - indicates no growth*
*Bgneg-bile tolerant gram negative bacteria, E.coli-Escehrichia coli, Sal-Salmonella species, Shig-Shigella Species, P.aue-Pseudomonasauruginosa S.aur-Staphylococcusaureus,Clost- Clostridia species and C.alb- Candida albicans. All test carried with positive microorganism.*

## DISCUSSION

From Table-1,it can be observed that gelatin, lactose, sodium alginate and xanthan gum are heavily contaminated with microorganisms,which is evident from theirMPNs(which is more than 1000 /gm). The sample thyroxine sodium and acacia have MPN in the range of 20 to 1000/gm. All other sampleshave MPN in the range of 11 to 3 /gm or ml**.**

After enrichment, Dried Aluminium hydroxide gel and thyroxine sodium shown growth only on violet red bile glucose agar and there was no similarity with positive control. Hence samples may be free from bile tolerant gram negative bacteria. The growth found(as mild pink colour colonies) in MacConkey broth forGelatin and Thyroxine sodium produced loss of violet colour of the medium and no growth was found for other raw materials anddid not produce any colour change. The identity of above said colonies have to be established.

The enrichment raw materials ofboth acacia and thyroxine sodium have shown only colour changes from pale green and growth observed on Xylose Lysine Deoxycholate agar, produces pale pink colony colour for acacia and yellow colour colonyfor thyroxine sodium. The absence of characteristic growth(a red coloured colony)and further confirmatory test negative for the prescenceof *Salmonella* species. The enriched GN Broth shown mild turbidity for all samples, but thyroxine sodium shown more turbidity, and growth was present on Xylose lysine Deoxycholate agar. The colony morphology and further confirmation test produces a negative result for S*higella* species.

In Mannitol salt agar, most of the samples produced growth as shown in the table: 2.Pink or near red colourcolonies were observed, which is not the characteristic growth pattern of S. aureus. The confirmatory test also indicated the absence of *Staphylococcus aureus*. The growth was not observed on both Cetrimide agar and Columbia agar, which indicates that all raw materials, are free from both *Pseudomonasaeruginosa* and *Clostridia* species. Only the Sabouraud dextrose broth of Xanthangum produced a pale white colour appearance on sabouraud dextrose agar and confirmatory test produced negative result for *Candida albicans*.

## CONCLUSION

From the above studies, in all the tested raw materials, gelatin, lactose, sodium alginate and xanthan gum had more number of microorganisms so it needs strict aseptic condition during processing of raw materials. Thyroxine sodium, Acacia, Dried Aluminium hydroxide gel, Gelatin, lactose, liquid paraffin, sodium alginate, starch and Xanthangum have shown growth on only few selective media and growth was absent in other samples. Even though the growth was found in particular selective media, none of the tested samples found to have any objectionable

microorganisms. But the entire studies required more than one week for completion, which is not acceptable by many Pharma industries, since this time delay may affect the manufacturing of many dosage forms and other related activities. So a fast and reliable protocol for microbiological examination of raw materials is essential(like MALDI-TOF,ATP Luminescence studies) in order to produce quality medicines.

**Acknowledgment**
The authors acknowledge the University Grants Commission, New Delhi for the grant under the Major Research Project.

## REFERENCES

[1] United States Pharmacopoeia. Microbiological examination of non-sterile products: Tests for specified microorganisms. United States Pharmacopoeial Convention, Twin brook, Parkway Rockvalie, 31st edition. **2012**,1,56-65.
[2] M Russell, In Microbial Quality Assurance in Cosmetics, Toiletries and Non-sterile Pharmaceuticals, Taylor & Francis, London **1996**,31-47.
[3] EJG Glencross, *Br. Med. J.* **1972**,2, 376-378.
[4] RL Berkelman, RL Anderson, BJ Davis, AK Highsmith,NJ Petersen, WW Bono,EH Cook, MS Mackel,MS Favero,and WJ Martone, *Appl. Environ. Microbial*,**1984**; 47,752-756.
[5] ME Aulton, Pharmaceutics-The design and manufacture of medicines, 3rd Eds., Churchill Livingstone, United Kingdom,**2007**, 640–649.
[6] SP Denyer, and RMBaird,Microbial contamination, spoilage and hazard.Guide to microbiological control in pharmaceuticals and medical devices: 2ndEds., Taylor & Francis, London, **2007**, 24-44.
[7] Indian Pharmacopoeia, Microbiological examination of Non-sterile products: Tests for specified microorganisms. The Indian Pharmacopoeia commission Central Indian pharmacopoeia laboratory Government. of India, Ministry of health & family welfare, Ghaziabad.**2014**,1,37-48.
[8] JL Oblinger and JA Goburger, *J Milk food Tech***1975**,38(9),540-545.
[9] CH Collins, PM Lyne, Microbiological methods, 5th Edition, Butterworth &co Ltd, United Kingdom, **1984**,137-140.
[10] Scott Sutton, *Journal of Validation Technology*, **2010**,16,35-38.
[11] RM Baird, NA Hodges and Denyer,Handbook of Microbiological Quality Control: Pharmaceuticals and MedicalDevices. S.P., Eds., Taylor & Francis,London,**2000**: 86-106.
[12] KR Aneje,Experiments in Microbiology, Plant Pathology and Biotechnology, 4th Revised Edition, New age International Publishers. New Delhi.**2003**,74-294.