



## Microbiological Appraisal of Three Different Brands of Commercially Available Irreversible Hydrocolloid Impression Materials: An *in vitro* Study

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### ABSTRACT

**Aim:** To evaluate factory-sealed containers of three different commercially available irreversible hydrocolloid impression materials for their microbial contamination and the type of microorganisms present.

**Materials and methods:** Thirty-six measured samples were taken of each brand and placed on chocolate agar plates or in thioglycolate broth tubes and were incubated along with appropriate parallel controls. After incubation, colonies were enumerated and identified using standard microbiological methods.

**Results:** The three brands contained viable microorganisms in 90% of the samples. Samples from the top, middle and bottom layers had approximately equal contamination frequencies. The concentration of organism varied from 25 to 74 CFUs per gram of contaminated sample.

**Conclusion and clinical significance:** The samples contained viable microorganism which may be potentially dangerous to immunocompromised patients.

**Keywords:** Microbiological, Appraisal, Sealed, Irreversible hydrocolloid, Immunocompromised, Impression material.

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### INTRODUCTION

Nowadays, there has been an increasing emphasis on infection control in dentistry because of proliferation of infectious diseases and increase in number of medically

compromised patients. Patients with HIV, cancer, transplants, autoimmune diseases are often immunocompromised and any microorganism they are exposed to must be considered as a potential pathogen. As it is impossible to determine the patients' immunological condition during each dental treatment, there is a need for standardized precautions and standards. The concept of universal precaution is advocated because neither all patients with infectious diseases are aware of their status nor can they be identified by medical history or physical examination. Therefore, it is important to prevent cross-contamination in every dental procedure.

The risk of transmitting pathogenic microorganisms via impressions has been considered as a topic of interest for a number of years. The possibility of contaminating immunocompromised patients in minor dental procedures, since they are susceptible to infections by microorganisms of low virulence, has been pointed out.<sup>1</sup> Impression procedures frequently cause bleeding from the oral tissues. Considering that blood is a rich culture and microbial transportation medium, and that any rupture of skin integrity offers an opening for the entrance of potentially pathogenic microorganisms, there is a risk of accidental transmission of this infectious substrate to undesired places.<sup>2</sup> Materials like irreversible hydrocolloid are often positioned against mucous membrane when used and could potentially result in inoculation of the patient. The use of contaminated impression materials may represent a potential hazard to immunocompromised patients.

The need for blocking this potentially infectious route between impression materials and patients is generally theoretical as information on the microbial contamination of impression materials is very scarce. The sterility of dental

materials as delivered to the dentist from manufacturers has very little information about infection control during processing and packaging. Several studies have addressed preventing the transmission of microorganisms via impression materials after exposure to the patient's oral fluids.<sup>3-6,16-18</sup> One aspect that has been neglected is the risk from contamination of dental materials during manufacturing and packaging process. Contamination standards on expendable dental materials are not available. Until today, very few measures have been taken by manufacturers to avoid such contamination.

Gates et al found bacterial contamination in all the tested sealed bottles of four commercially available denture adhesives.<sup>7</sup> Moghadam et al found aerobic bacterial contamination in approximately 30% of the samples in four dental materials.<sup>8</sup> After that, other studies were performed on irreversible hydrocolloid pointing out the contamination of various brands, including those containing antimicrobial agents.<sup>9-11.</sup>

The purpose of this study was to evaluate factory-sealed containers of three different commercially available irreversible hydrocolloid impression materials for their microbial contamination and the type of microorganisms present.

## MATERIALS AND METHODS

The study was conducted using testing equipment in the Department of Microbiology, JNMC, Belgaum and Department of Prosthodontics and Crown and Bridge, KLE's Institute of Dental Sciences, Belgaum. Three sealed packets, each of three different brands of most commonly used commercially available irreversible hydrocolloid impression materials, were purchased from the manufacturers. The tested materials were hydrogum (Batch No. A0243B, Heraeus Kulzer, Germany), plastalgin (Batch No. 08801623001, Septodont, Italy), algitex (Batch

No.Y3213, DPI, India) (Fig. 1). The packaging of all the packets was examined to ensure that they were free from any violation.

With full aseptic precautions, the sealed packets were opened in a laminar flow hood and four samples measuring 0.06 gm each were removed from the top, middle and bottom layers with the help of sterile instruments. Individual chocolate agar plates (for culture of aerobic microorganisms) were then inoculated by streaking procedure with six samples from each packet; two from the top, two from the middle and two from the bottom. The remaining samples were placed in tubes of thioglycolate broth (for culture of anaerobic microorganisms) and were vortexed for 10 seconds to disperse the irreversible hydrocolloid. For each sample, appropriate parallel controls of uninoculated chocolate agar plates and the same number of uninoculated thioglycolate broths served as negative controls.

Ten chocolate agar plates and 10 thioglycolate broth tubes were challenged with a mixture of four isolates (*E. coli* ATCC No. 25922, *Staphylococcus aureus* ATCC No. 29213, *Pseudomonas aeruginosa* ATCC No. 27853, and *Streptococcus faecalis* ATCC No. 29212) to ensure that the media supported growth of microorganisms.<sup>12</sup>

All chocolate agar plates were labeled individually according to the tested material and placed in an incubator at 37°C for 7 days. After 7 days, they were observed for presence or absence of microbial growth (Fig. 2). The thioglycolate broths inoculated with samples were incubated at 37°C for 5 days in an incubator. After 5 days, any sample showing turbidity was again subcultured to chocolate agar and blood agar plates which were kept in McIntosh Fildes jar and incubated for two days at 37°C in an incubator (Fig. 3).

After incubation, the colony forming units (CFU) were counted based on the values obtained for 0.06 gm and the organisms were identified based on colonial morphology, Gram's stain, coagulase test, hemolytic reactions and pigmentations.



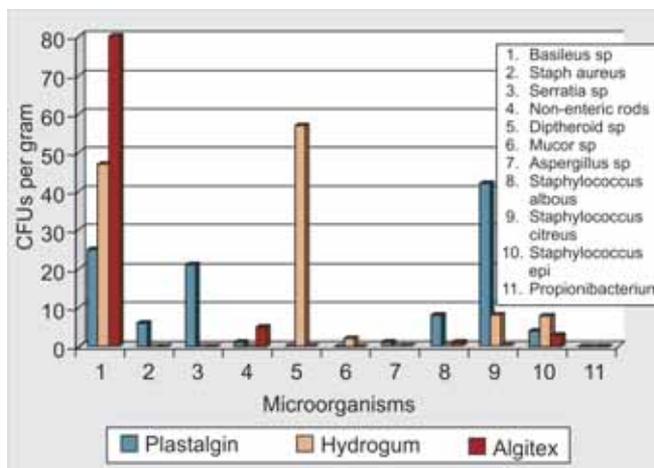
Fig. 1: Materials used



Fig. 2: Chocolate agar plates (before and after culture)



Fig. 3: Thioglycolate broth (before and after culture)



Graph 1: Chocolate agar contamination concentration for each brand (number of CFUs per gram)

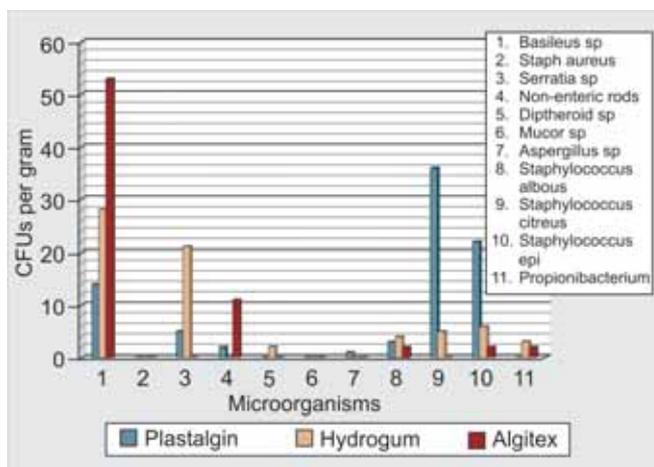
The number of positive results in each category of media, the location of the sample, and the brand was divided by the total number of samples to arrive at the contamination frequency. This factor was compared with the data from the control groups and other experimental groups using Student’s t-test for statistical significance to determine the results.

**RESULTS**

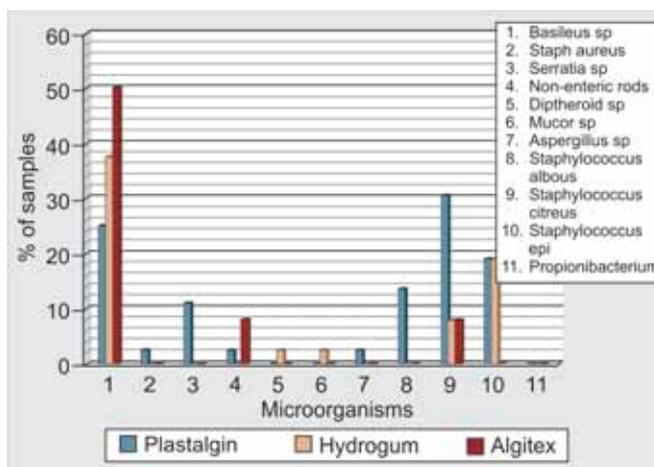
All culture media inoculated as positive controls with microorganisms exhibited appropriate growth. All uninoculated culture media were void of growth. None of the negative controls exhibited any growth.

There was an average of 44 CFUs per gram of contaminated material. Plastalgin showed the least at 25 CFUs per gram, followed by hydrogum which had 32 CFUs per gram and algitex at 74 CFUs per gram. The number of CFUs per gram of the sample in chocolate agar plates and thioglycolate broth tubes is given in Graphs 1 and 2.

The spectrum of various microorganisms in chocolate agar and thioglycolate broths is shown in Graphs 3 and 4. Inoculated chocolate agar plates showed an average contamination frequency of 96.2% while that of thioglycolate broth tubes resulted in a contamination average of 82.8%, the difference was not statistically significant. There was no significant difference in contamination frequency between the top, middle and bottom layers of samples from the packets (89.8, 88.6 and 93% respectively) as shown in Table 1. The overall contamination average (top, middle and bottom; both chocolate agar and thioglycolate broth) was plastalgin—88%, algitex—94.4%, hydrogum—88% (Table 2).

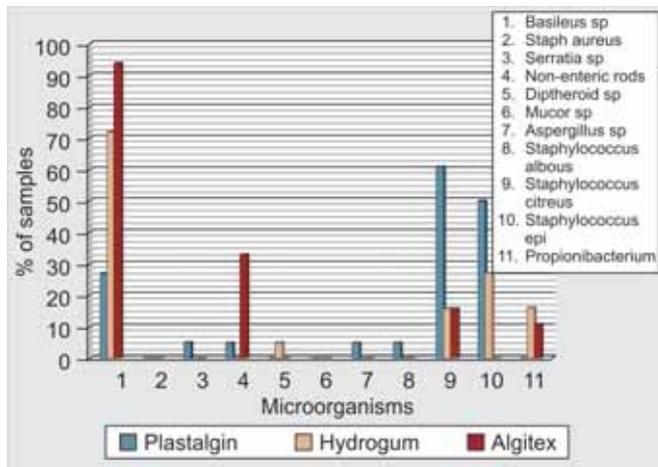


Graph 2: Thioglycolate broth contamination concentration for each brand (number of CFUs per gram)



Graph 3: Chocolate agar contamination frequency (%)

saliva or blood from contaminated impression material, particularly irreversible hydrocolloid impression material. Irreversible hydrocolloid impression material is forcefully positioned against oral mucosa while making the impression.



Graph 4: Thioglycolate broth contamination frequency (%)

Table 1: Comparison of contamination frequencies (%) at different layers of the three brands (both chocolate agar and thioglycolate broth media)

Layer	Brand			Total
	Plastalgin	AlgiteX	Hydrogum	
Top	87.5%	92.5%	89.7%	89.8%
Middle	88.2%	90.4%	88.1%	88.6%
Bottom	92%	95.4%	93.4%	93.2%

Table 2: Overall contamination percentage (both chocolate agar and thioglycolate broth)

	Brand		
	Plastalgin	AlgiteX	Hydrogum
% of contaminated samples	88%	94.4%	88%

A visual study of the impression immediately after removal from the mouth often reveals blood clinging to the material.<sup>13</sup> Blood is a well-recognized vector of pathogenic microorganisms and there are few if any dental procedures in which blood contamination is not a risk.<sup>14</sup> There is a chance of potentially inoculating microorganisms if the material is contaminated. Because alginates have polysaccharide structures similar to those of agar which is an excellent substrate for microorganisms, it seems unlikely that alginate powders would be free from microorganisms. Hence, the use of contaminated impression materials may represent an additional risk of inoculation of microorganisms and, consequently, of occurrence of diseases in immunocompromised patients. Though various studies have shown that irreversible hydrocolloids may contain viable microorganisms; however, very few measures have been taken by manufacturers to address this issue.<sup>9-11,15,19</sup> No contamination standards are known to exist for expendable dental materials. There is very little information regarding the sterility of dental materials as delivered to the dentist

from the manufacturer. Sterilization methods should be incorporated, such as irradiation with gamma rays at the end of production. Nonetheless, such method requires a previous knowledge concerning microbial load, both qualitatively and quantitatively, in order to determine the doses to be applied. To improve the basis for risk assessment, and to find a suitable strategy for cross-contamination risks, there is a need to study to determine microbial contamination in impression materials, particularly alginate.

Murray found all study samples contaminated with microorganisms which could pose danger to immunocompromised patients.<sup>16</sup> Moghadam et al studied incidence of aerobic bacterial contamination in 12 different dental materials and found viable organisms in samples of 4 of 12 different materials.<sup>8</sup> Rice compared four brands of irreversible hydrocolloid impression materials and concluded that viable organisms were present in 50% to 100% of the samples.<sup>9</sup> In another study by Rice on bacterial contamination of unopened packets of irreversible hydrocolloid impression material and gingival retraction cord, a statistically significant number of alginate samples were found to be microbiologically contaminated.<sup>10</sup> Rice et al also studied antimicrobial alginates for contamination and found that 12.5% of the samples contained viable organisms.<sup>11</sup>

A statistically significant (90%) percentage of samples from the three brands of irreversible hydrocolloid impression material tested showed microbial contamination irrespective of the brand or batch. This is in agreement with findings of Rice et al who found viable organisms in 100% of the samples.<sup>9,11</sup> The chocolate agar media showed an average contamination frequency of 96.2% of samples, while that of thioglycolate broths showed a contamination average of 82.8%. This may have been due to the clumping of irreversible hydrocolloid in the thioglycolate broths.

With respect to the microorganisms isolated, the spectrum of contaminants in chocolate agar found *Bacillus sp* (72%) which are common environmental contaminants of low virulence. These were followed by *Staphylococcus citreus* (31%), *Staphylococcus epidermidis* (26%), *Staphylococcus albus* (9%), *Serratia sp* (7.4%) (Fig. 4), *Mucor* (1.8%) and *Diphtheroids* (1.8%) which are opportunistic pathogens. These findings are similar to those detected in studies carried out by Rice and Gates.<sup>9,11,7</sup> *Staphylococcus aureus* (1.8%) and *Aspergillus* (1.8%) which are moderately virulent organisms were also isolated and have been documented as a cause of infection in immunocompromised patients.<sup>13</sup> Similar findings were seen in studies by Rice, Powell and Montgomery who found these organisms in less than 1% of the samples.<sup>9,17,18</sup>



**Fig. 4:** *Serratia* species (gram-negative bacilli)

Organisms detected in the thioglycolate broths were *Bacillus sp* (65%), *Staphylococcus citreus* (31%), *Staphylococcus epidermidis* (26%), *Staphylococcus albus* (1.8%), Nonenteric gram-positive rods (13%) and *Aspergillus* (1.8%) all discussed previously. Propionibacterium (9%) which is an anaerobic skin commensal of low virulence was also found.

Colony forming units per gram of impression material was also measured and an average of 44 CFUs/gm of irreversible hydrocolloid impression material was found, while a study by Rice et al found 12 to 82 CFUs/gm.<sup>9</sup> Algitec showed the highest CFUs/gm at 74, followed by hydrogum at 32 CFUs/gm and plastalgin at 25 CFUs/gm. The difference found was not statistically significant. Overall, the percentage of contamination for each brand was 94.4% for algitec, 88% for hydrogum and 88% for plastalgin.

Regarding the layer-wise comparison of contamination frequency, no significant difference was found in the frequency of contamination in the top, middle and bottom layers of the irreversible hydrocolloid packets respectively. This finding is again in accordance with studies by Rice et al.<sup>9,11</sup>

### CLINICAL SIGNIFICANCE

Clinically, the average clinical material required for an alginate impression is 18 gms which is 300 times the testing sample. This would yield an average concentration of 1200 organisms per average clinical sample of material for all the materials combined which is higher than in previous studies.<sup>9,11</sup>

Most of the organisms found were avirulent members of the environmental flora. McGhee et al cautioned that the compromised patient may have an altered flora and be susceptible to infection with less usual organisms and ordinary dental procedures. In severely immunocompromised hosts, organisms that are considered avirulent

could nonetheless be pathogenic. A small percentage of samples were found to contain virulent organisms that may represent a possible risk to all patients. Routine use of contaminated dental materials in immunocompromised patients could lead to inoculation.

With regards to the origin of the contamination present, it may be due to the materials used, to the manufacturing process, transportation, and even caused iatrogenically. This may be due to the fact that, even if the alginates were free of contamination at first, there would certainly still be a possibility of contamination because the material is kept in a large package, whose content is sufficient to perform many impressions. By opening the container, microorganisms from the environment could be introduced into it, which would make the material inadequate for further use from a microbiological point of view.

### CONCLUSION

All the irreversible hydrocolloids tested showed microbiological contaminants with brands varying appreciably in spectrum and concentration of contaminants. Most of the organisms were low virulent contaminants that may represent a hazard to immunocompromised patients. A small percentage of samples were found to contain virulent organisms that may represent a possible risk to all patients. More attention should be paid to the microbiological quality and sterility of commercially available alginates and to determine, by performing other studies, if these materials should be sterile or should be allowed a microbiological limit, both qualitatively and quantitatively. It is also recommended that the packages be presented with a single-use size (for a single impression) in order to preserve the microbiological quality of the material.<sup>19</sup>

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