Plaque Inhibition:
The Science and Application of Oral Rinses

Authored by Richard Demke, DDS

Upon successful completion of this CE activity 2 CE credit hours may be awarded
LEARNING OBJECTIVES
After participating in this CE activity, the individual will learn:
• The fundamentals of plaque biofilm and the role of oral rinses in plaque inhibition.
• Plaque control and treatment options currently available.

ABOUT THE AUTHOR
Dr. Demke received his DDS degree from Marquette Dental School, and BS degree in science from the University of Wisconsin. He maintained a private practice in central Wisconsin and created a company to commercialize dental education booklets he wrote and produced. He spent 2 years with Siemens in the development of nuclear medicine devices, and 13 years at GC America as the vice president of research and development. He can be reached at richard.demke@us.sunstar.com.

Disclosure: Dr. Demke is currently the senior director of technology and new product development for Sunstar Americas.

INTRODUCTION
The purpose of this article is to inform dental clinicians about the fundamentals of plaque biofilm, at-home treatment and control options currently available, the role of oral health rinses, and how and why mouthrinses should be applied in daily practice to provide more effective treatment and improved outcomes regarding periodontal disease.

THE PREVALENCE OF PERIODONTAL DISEASE
The prevalence of all forms of periodontal disease in the United States is approximately 75%,1 and recent research by the American Academy of Periodontology and US Centers for Disease Control and Prevention suggests that periodontal disease prevalence rates may have been underestimated by as much as 50%.1

Periodontal disease is a bacterial infection known to be caused by the effects of certain pathogenic bacteria that colonize the oral cavity. The early form of periodontal disease, gingivitis, has the potential to cause gingival inflammation and bleeding. Gingivitis is plaque induced and initiated by a host-inflammatory response to maturing plaque biofilm, which is the primary etiological factor for gingival inflammation.2 If left untreated, the disease may progress to periodontitis, which may lead to tissue recession, bone loss, and tooth loss. Advanced stages of periodontitis have been linked to systemic diseases, such as cardiovascular disease and diabetes.3,5

Dental biofilm is a living community of bacteria comprised of millions of cells that possess vigorous metabolic and reproductive attributes. Biofilm grows as a complex, 3-dimensional, self-protective, and sticky structure that is built up in layers (Figure 1). Biofilm consists of different species of bacteria, notably Actinobacillus actinomycetemcomitans, Streptococcus mutans, Fusobacterium nucleatum, Treponema denticola, Porphyromonas gingivalis, and Tannerella forsythus.6-8 These species of bacteria are built up sequentially in layers, which enable them to interact and cooperate with each other using various forms of communication. Chemical signals are the most common method; however, electrical signals and the exchange of genetic material have also been documented. The signals are specific, organized, and timed according to conditions in the oral cavity that favor colonization of subsequent bacterial species (Figure 2). These bacteria facilitate the arrival of other bacteria by providing diverse adhesion sites, called co-adhesion and co-aggregation.6-8 Some species are not able to attach to a surface on their own, but are often able to anchor themselves to the biofilm matrix or directly to other bacteria. Once a biofilm colony has formed in the mouth, it releases chemicals and enzymes that signal other bacteria to join the colony.6

The challenge, therefore, is to disrupt the colonization, proliferation, and sequential layering of biofilm in order to interrupt the development and progression of periodontal disease.9 During the past 200 years, various means have been devised with varying degrees of success, which is largely determined by the effectiveness of the patient's oral care regimen.
CURRENT TOOLS AND RESOURCES TO INHIBIT PLAQUE

Perhaps the most common means of plaque control, or more specifically, biofilm disruption, has been the daily and proper use of a toothbrush. William Addis of England is believed to have produced the first mass-produced toothbrush in 1780, and the first American to patent a toothbrush was H.N. Wadsworth in 1857. Toothbrushes, however, are not always effective in cleaning interproximal surfaces, as these areas are beyond the reach of most toothbrush bristles. In addition, gingival sulci are often not cleaned as well as desired due to the individual's brushing technique.

Dental floss has been proven highly effective in disrupting biofilm in interproximal areas, including portions of the sulcus; however, its effectiveness is technique sensitive and dependent on the patient's skill, frequency of use, and motivation. The ADA statistics indicate that only 11% to 51% of people in developed countries claim to use dental floss or some other interdental cleaning device.

Another time-tested plaque removal implement is the wooden toothpick. Recent advances in synthetic materials and design have provided new soft picks and nylon bristle brushes, which improve the ability to access tight interproximal spaces. These advancements also caused their name to change to “interdental cleaners,” which is more descriptive of their function. Interdental cleaners can be effective adjuncts to other mechanical means of biofilm disruption if used correctly and regularly. Some interdental cleaners have rows of soft, tiny bristles that can break up and sweep away supragingival biofilm between teeth, around and under fixed prosthetic and orthodontic appliances. Examples of interdental cleaners featuring small bristles are GUM Go-Betweens Proxabrush Cleaners (Sunstar Americas), and the Oral-B Interdental Brush System (Oral-B).

All of the aforementioned devices are useful and effective on hard tissues and, to a lesser extent, gingiva. However, due to their designs, these devices are unsuitable for cleaning the sensitive oral mucosa, which can harbor more bacteria than teeth and gums combined. Mechanical methods of oral hygiene are targeted only toward tooth surfaces and the dentogingival margins (approximately 10% to 20% of the total oral surface area), and do not act on the mucosal surfaces of the oral cavity. Therefore, the remaining 80% to 90% of uncleaned soft tissues can rapidly reseed the mechanically cleaned areas with their ample reserves of living bacteria; unstimulated saliva contains 50 million to 100 million bacteria per mL. Therefore, it is necessary to address these large bacterial reserves in order to reduce or eliminate their potential to rapidly repopulate the teeth and gingival surfaces.
Advent of Mouthrinse

In 1879, Drs. Joseph Lawrence and Jordan Wheat Lambert developed a liquid antiseptic formula for use as a surgical disinfectant and named it after Sir Joseph Lister, an English physician famous for performing the first antiseptic surgery in 1865. In 1884, the Lambert Company began to manufacture and market LISTERINE products to the medical community as a multipurpose antiseptic. In 1895, the Lambert Company extended the sale of LISTERINE disinfectant to the dental profession as a powerful oral antiseptic. By 1914, the formula had become popular and was one of the first prescription products to be available over the counter, thereby founding the mouthwash category.20 In the decades following, other manufacturers of oral care products developed cosmetic oral rinses that primarily freshened breath via antiseptic and flavoring agents, but in the 1970s a separate classification of mouthrinses, called therapeutic mouthrinses, was established.

ROLE OF THERAPEUTIC MOUTHRINSES

Ongoing research in oral bacteriology has resulted in greater understanding of the ways aerobic and anaerobic bacteria enter the mouth, colonize, reproduce, communicate, and mutate. Therapeutic mouthrinses use various active ingredients to support the key benefits offered, and are available over the counter and by prescription. Primary active ingredients of therapeutic mouthrinses include:

1. A fixed mixture of essential oils (phenol compounds)
2. Quaternary ammonium compounds (cetylpyridinium chloride [CPC])
3. Bisbiguanide antiseptics (chlorhexidine gluconate [CHX]) by prescription only in the US and Canada.
4. Amine alcohols (delmopinol hydrochloride).

The active ingredients in therapeutic mouthrinses may control oral biofilm at differing stages of colonization, which includes colonization of the soft tissues; however, therapeutic mouthrinses are most effective when used in combination with other oral care procedures. Mouthrinses are not intended to replace mechanical attempts to remove/disrupt organisms and colonies on tooth surfaces and gums, but rather to provide an adjunctive means to achieve greater and more consistent reductions in plaque and gingival inflammation.

Therapeutic mouthrinses provide an additional level of effective care adjunctive to toothbrushing, interproximal cleaning, and prophylaxis. In support of this, the rationale for daily use of antiplaque mouthrinses is twofold: (1) as a component added adjunctively to mechanical oral hygiene regimens for the control and prevention of gingivitis, and (2) as a method for delivering antiplaque agents to mucosal sites throughout the mouth that harbor pathogenic bacteria capable of recolonizing supragingival and subgingival tooth surfaces.21 The properties of antiplaque agents can be bactericidal, bacteriostatic, or antiadhesive, which may prevent plaque from developing on clean tooth surfaces.

EFFICACY AND EFFICIENCY OF ORAL HEALTH RINSES IN CONTROLLING PLAQUE AND GINGIVITIS

Therapeutic mouthrinses provide a variety of choices that

Figure 3. (a) Placebo: tooth brushing, topical application of placebo, no toothpaste, plaque disclosing. (b) Including delmopinol: tooth brushing, topical application of delmopinol, toothpaste, plaque disclosing. (Photos courtesy of Dr. Richard Nagelberg.)

Figure 4. Periodontitis is a chronic, noncurable bacterial infection. Failure to prevent the progression of gingivitis (a) to periodontitis (b) condemns the patient to a lifetime of disease management. (Photo courtesy of Dr. Richard Nagelberg.)
approach plaque control through different strategies to interrupt and/or reduce the proliferation of pathogenic bacteria and the progression of periodontal disease. These strategies range from adjunctive oral health maintenance to acute disease intervention. Therefore, the choice of mouthrinse may be informed and guided by the patient’s clinical needs and tolerance of the formulation’s active and inactive ingredients.

1. Essential oils/phenols. The most well-known mouthrinse with a phenol-related fixed mixture of essential oils formulation is LISTERINE (Johnson & Johnson). It contains Thymol (0.064%), eucalyptol (0.092%), menthol (0.042%), and methyl salicylate (0.060%). This combination is dispersed in a denatured alcohol vehicle (between 21.6% to 26.9%).

The mechanism of action is complex. At high concentrations, there is a disruption of the cell wall and precipitation of cell proteins; at lower concentrations, essential (bacterial) enzymes are inhibited. This formulation can penetrate plaque biofilm and exert bactericidal activity. The bacterial load is reduced with concomitant decrease in plaque mass and pathogenicity.22

2. Quaternary ammonium compounds (CPC). CPC is a cationic (positively charged) surface-active agent that has a broad antimicrobial spectrum of activity that involves the rapid destruction of Gram-positive pathogens and yeasts. An example of a CPC-containing therapeutic mouthrinse is Crest Pro-Health Multi-Protection Rinse (Procter & Gamble). Crest Pro-Health contains high bioavailable CPC (0.07%) in

Table 1. Indications and Contraindications for 4 Representative Products as set Forth by Their Respective Manufacturers

<table>
<thead>
<tr>
<th>Product Name</th>
<th>LISTERINE Antiseptic</th>
<th>Crest Pro-Health Multi-Protection Rinse</th>
<th>PERIDEX Chlorhexidine Gluconate (CHX) 0.12% Oral Rinse</th>
<th>GUM PerioShield Oral Health Rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Ingredient(s)</td>
<td>Fixed combination of 4 essential oils(^a)</td>
<td>Quaternary ammonium compounds/cetylpyridinium chloride 0.07%</td>
<td>CHX 0.12%</td>
<td>Amine alcohols/delmopinol hydrochloride 0.2%</td>
</tr>
<tr>
<td>Primary Mode of Action</td>
<td>Antimicrobial</td>
<td>Antimicrobial</td>
<td>Antimicrobial</td>
<td>Antiadherent</td>
</tr>
<tr>
<td>Indications</td>
<td>Kills germs that cause bad breath, plaque, and gingivitis.</td>
<td>Antigingivitis/antiplaque rinse; fights bad breath; alcohol free.</td>
<td>For use between dental visits as part of a professional program for the treatment of gingivitis as characterized by redness and swelling of the gingivae, including gingival bleeding upon probing.(^b)</td>
<td>Helps prevent and treat gingivitis; recommended for patients with heavy plaque and chronic gum inflammation.</td>
</tr>
<tr>
<td>Contraindications</td>
<td>Not for patients younger than age 6 years.</td>
<td>Not for patients younger than age 12 years.</td>
<td>Should not be used by persons who are known to be hypersensitive to CHX, or other formula ingredients.</td>
<td>Individuals with known hypersensitivity to any of the ingredients; children under age 12 years or pregnant women.(^c)</td>
</tr>
<tr>
<td>Adverse Reactions</td>
<td></td>
<td></td>
<td>Increase in staining of teeth and other oral surfaces; increase in calculus formation; alteration of taste perception.</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Thymol 0.064%, eucalyptol 0.092%, methyl salicylate 0.060%, menthol 0.042%, solubilized in 21.6% to 26.9% denatured alcohol.

\(^b\) CHX Oral Rinse has not been tested among patients with acute necrotizing ulcerative gingivitis.

\(^c\) Due to limited testing of this product on these populations.
an alcohol-free formulation.

The mode of action is through the disruption of the cell membrane function, leakage of cytoplasmic material, and collapse of intracellular equilibrium. The substantivity (or duration of effectiveness) of CPC is reported to be between 3 and 5 hours, at least in part due to its cationic nature.23

It is significant to note that not all CPC-containing mouthrinses provide the same degree of clinical benefit due to the bioavailability of CPC. The formulation of the vehicle ingredients can have a significant impact on the bioavailability of CPC. Increased bioavailability is associated with higher probability of effectiveness, greater antiplaque activity, and greater reductions in gingivitis; decreased bioavailability and lower concentrations of CPC are associated with cosmetic claims alone, such as in vitro germ killing (and) fresh breath.19

3. Bisbiguanide antiseptics (CHX). CHX has been widely used in medicine and surgery for presurgical disinfection since the 1940s, and was first investigated for effectiveness in the oral cavity in 1970.24 It is considered the “gold standard” therapeutic mouthrinse. Originally, this prescription-only mouthrinse (in the US and Canada) was formulated with alcohol (approximately 11.6%), and now alcohol-free formulations are available. An example of an alcohol-containing CHX 0.12% mouthrinse is PERIDEX (3M ESPE), while an example of an alcohol-free CHX 0.12% mouthrinse is GUM CHX Oral Rinse USP, 0.12% (Sunstar Americas). The CHX molecule is a strong base, with 2 positive charges (dicationic) at pH levels greater than 3.5.25 These 2 positive charges make CHX extremely interactive with anions and are the basis of its clinical effectiveness as well as its unwanted effects, such as tooth staining.

The primary mode of action is thought to occur against the cell wall, which is negatively charged. The positively charged CHX molecule is rapidly attracted to the negatively charged cell surface, and binds via adsorption to oral surfaces, the pellicle, and saliva. At low concentrations, the integrity of the cell membrane is altered and leads to increased permeability and leakage of low molecular weight intracellular contents, but this is reversible and the cell can recover. This effect is considered bacteriostatic. At higher concentrations, there is

### Table 2. Comparison of Plaque and Gingivitis Scores Among 4 Representative Mouthrinse Types

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Maximum Plaque Reduction (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Maximum Gingivitis Reduction (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Maximum Bleeding on Probing Reduction (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine 0.12%</td>
<td>60.9%</td>
<td>42.5%</td>
<td>77%</td>
<td>30, 31, 35, 39</td>
</tr>
<tr>
<td>Fixed Combination of Essential Oils&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.3%</td>
<td>35.9%</td>
<td>69.8%</td>
<td>11, 31-35, 37, 39</td>
</tr>
<tr>
<td>Delmopinol Hydrochloride 0.2%</td>
<td>35%</td>
<td>18%</td>
<td>57%</td>
<td>29, 35, 37, 38</td>
</tr>
<tr>
<td>Cetylpyridinium Chloride 0.07%</td>
<td>15.8%</td>
<td>15.4%</td>
<td>33.3%</td>
<td>36</td>
</tr>
</tbody>
</table>

<sup>a</sup> Comparison between agents is inadvisable due to differences in study design and indices reported.

<sup>b</sup> Compared with negative control at 6 months.

<sup>c</sup> Thymol 0.064%, eucalyptol 0.092%, methyl salicylate 0.060%, menthol 0.042%, solubilized in 21.6% to 26.9% denatured alcohol.
reduced leakage of low molecular weight intracellular contents, but coagulation along with precipitation of the cytoplasm occurs, which is irreversible and therefore the effect is considered bactericidal.\textsuperscript{26}

4. Amine alcohols (delmopinol hydrochloride). A new generation of antiplaque and antigingivitis agents has been established that inhibits or disrupts the formation of plaque while possessing little, if any, effect on the bacteria, thus avoiding disruption to the balance of bacterial flora found in a healthy mouth. Delmopinol hydrochloride (morpholinoethanol derivative) is an amine alcohol that functions as a surface-active agent shown to interact with pellicle constituents and inhibit glucan synthesis by \textit{S. mutans}.\textsuperscript{27} It has little or no demonstrable effect on the bacteria, but it interferes with plaque/biofilm matrix formation. The nascent biofilm mass, being loosely adherent, produces a reduction in the proportion of dextran-producing cocci.\textsuperscript{28} The interference with plaque matrix formation leads to the plaque deposit being less sticky and less dense, which makes it easier to remove through mechanical means.

An example of a delmopinol hydrochloride oral rinse is GUM PerioShield Oral Health Rinse (Sunstar Americas). PerioShield contains delmopinol hydrochloride (0.2\%), which studies have shown to be effective against plaque and gingivitis in short-term studies on individuals with no oral hygiene, as well as in long-term, home-use studies. The short-term studies on individuals with no oral hygiene showed plaque inhibition close to that of CHX.\textsuperscript{29} (Figures 3a to 4b) (Tables 1 and 21,29-39).

**ORAL HEALTH RINSES RECOMMENDED ACCORDING TO PATIENT NEEDS**

The clinician's recommendation of a therapeutic mouthrinse is determined by the patient's clinical needs and tolerance of the product's ingredients, including sensory perceptions, as this can directly affect patient compliance. In general, the choice of mouthrinse may be based upon the patient's state of oral health. For example, for patients with acute gingivitis, the clinician may prescribe a CHX rinse, typically for 2 to 4 weeks. As the patient responds and oral health improves, the clinician may recommend a nonCHX rinse, such as a mixture of essential oils or CPC formulation if bacterial control is still desired, or a delmopinol rinse to inhibit the adhesion of plaque and facilitate easier removal. As a nonantimicrobial rinse, delmopinol helps maintain healthy oral flora, which may prevent potential overgrowths of antimicrobial-resistant strains. This property enables long-term use of delmopinol following short-term treatment with CHX, as well as treatment without CHX for less severe gingivitis patients where a strong antibacterial effect may not be indicated.

**Patient Acceptance**

After ruling out allergic reactions to the ingredients of any mouthrinse, the goal of the clinician is to recommend a mouthrinse that is: (1) effective for the treatment of the patient's condition, (2) tolerated by the patient for the recommended duration and frequency of use, and (3) has acceptable side effects.

The patient's sensory perceptions play a key role in acceptance; therefore, the clinician should anticipate the possible rejection of a recommended product based on its odor, flavor, inclusion of certain ingredients, or "mouth feel" while being used. For example, the presence of alcohol in a product may or may not be a factor in patient acceptance. Since alcohol is of no therapeutic benefit, alcohol containing formulations may be undesirable for diabetics, nursing mothers, alcoholics, and those who choose to avoid alcohol for any reason. For such patients, acceptance and compliance may be improved by recommending alcohol-free formulations.

In addition, tooth staining, taste alteration, stinging, and tongue numbing are factors that should be considered in regard to patient acceptance. While some patients may consider these effects to be incidental, easily tolerated, or merely temporarily bothersome, others may consider them unacceptable. Therefore the clinician should be prepared to recommend a different formulation when the patient's sensory perceptions or negative experience with a product threaten compliance.

**SUMMARY AND CONCLUSION**

The adjunctive use of therapeutic mouthrinses provides a way of overcoming deficiencies in mechanical tooth cleaning. Through direct destruction of susceptible oral bacteria or through the prevention of bacterial adhesion and aggregation,
therapeutic mouthrinses are a well-accepted means of interrupting the accumulation and progression of oral biofilms, which in turn may interrupt or prevent the progression of gingivitis. Therefore, therapeutic mouthrinses play an important role in the treatment and prevention of gum disease and in the maintenance of oral health.

REFERENCES


37. Lang NP, Hase JC, Grassi M, et al. Plaque formation and gingivitis after supervised mouthrinsing with 0.2% delmopinol hydrochloride, 0.2% chlorhexidine digluconate and placebo for 6 months. Oral Dis. 1998;4:105-113.


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POST EXAMINATION QUESTIONS

1. According the American Academy of Periodontology, the prevalence of periodontal disease in the United States is approximately:
   a. 25%.
   b. 40%.
   c. 50%.
   d. 75%.

2. Dental biofilm is composed of:
   a. Individual free-floating bacteria on the pellicle.
   b. One or more layers of bacteria attached to teeth and soft tissue.
   c. Calculus.
   d. Both a and c.

3. Adequate tooth brushing and flossing may clean up to ______ of the total oral surfaces:
   a. 20%.
   b. 50%.
   c. 80%.
   d. 40%.

4. Unstimulated saliva contains:
   a. 10 million to 50 million bacteria per liter.
   b. 25 million to 50 million bacteria per mL.
   c. 50 million to 100 million bacteria per mL.
   d. 75 million to 150 million bacteria per liter.

5. Certain therapeutic mouthrinses can:
   a. Kill all bacteria in the oral cavity.
   b. Temporarily attenuate the growth of oral bacteria.
   c. Prevent the attachment and layering of biofilm.
   d. Both b and c.

6. Therapeutic mouthrinses:
   a. Can replace mechanical methods of plaque removal.
   b. Are used as an adjunctive means to reduce plaque.
   c. Have no side effects when used as directed.
   d. All require a prescription.

7. Therapeutic mouthrinses:
   a. May control the growth or layering of free-floating and organized biofilm.
   b. May reach all tissues in the oral cavity.
   c. Achieve more consistent reductions in plaque.
   d. All of the above.

8. The rationale for using therapeutic mouthrinses is/are:
   a. So the patient may omit flossing when mouthrinses are used as directed.
   b. To augment mechanical oral hygiene regimens and deliver antiplaque agents.
   c. To allow for the nonsurgical treatment of periodontitis.
   d. Both b and c.
9. Therapeutic mouthrinses with a fixed mixture of essential oils:
   a. Are bactericidal when used as directed.
   b. Prevent layering of biofilm when used as directed.
   c. Are bacteriostatic when used as directed.
   d. May stain teeth when used as directed.

10. Therapeutic mouthrinses with high bioavailable cetylpyridinium chloride:
    a. Prevent layering of biofilm when used as directed.
    b. Are bactericidal and bacteriostatic when used as directed.
    c. Require a prescription from a doctor.
    d. Are bacteriostatic when used as directed.

11. Therapeutic mouthrinses with chlorhexidine gluconate (CHX):
    a. Is the “gold standard” therapeutic mouthrinse.
    b. Contains a strong base with 2 positive charges at pH levels above 3.5.
    c. Are extremely interactive with anions.
    d. All of the above.

12. Therapeutic mouthrinses with CHX:
    a. May be used indefinitely when used as directed.
    b. Prevent the attachment and layering of oral biofilm.
    c. Bind to the cell walls, which initiates its antibacterial mode of action.
    d. Do not require a doctor’s prescription in the United States.

13. Therapeutic mouthrinses with delmopinol hydrochloride:
    a. Prevent the attachment and layering of oral biofilm.
    b. Are bactericidal and bacteriostatic when used as directed.
    c. Disrupt the balance of oral flora.
    d. May not be used indefinitely when used as directed.

14. Therapeutic mouthrinses with delmopinol hydrochloride:
    a. Have little or no demonstrable effect on oral bacteria.
    b. Interfere with plaque/biofilm matrix formation.
    c. Make plaque less sticky and easier to remove.
    d. All of the above.

15. When should a dental professional recommend a therapeutic mouthrinse?
    a. When a patient’s efforts at mechanical plaque removal are inadequate.
    b. Not until a patient has periodontitis.
    c. When a patient has poor plaque control and is under the age of 6 years.
    d. Both b and c.

16. Therapeutic mouthrinses may work through:
    a. Inoculation of the oral cavity against microorganisms.
    b. Direct destruction of susceptible oral bacteria.
    c. Prevention of bacterial adhesion.
    d. Both b and c.
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5. ☐ a ☐ b ☐ c ☐ d 13. ☐ a ☐ b ☐ c ☐ d
6. ☐ a ☐ b ☐ c ☐ d 14. ☐ a ☐ b ☐ c ☐ d
7. ☐ a ☐ b ☐ c ☐ d 15. ☐ a ☐ b ☐ c ☐ d
8. ☐ a ☐ b ☐ c ☐ d 16. ☐ a ☐ b ☐ c ☐ d

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