How Useful Is Root Canal Culturing in Predicting Treatment Outcome?

Chankhrit Sathorn, DClinDent, Peter Parashos, PhD, and Harold H. Messer, PhD

Abstract

Microbial control—fundamental to healing of apical periodontitis—is central to endodontic practice. The effectiveness of antibacterial measures is generally monitored (in clinical research studies) by microbiological root canal sampling (MRS), which is often used as a predictor for healing. This article addresses the question of the extent to which positive or negative cultures at time of obturation are able to predict treatment outcome. To date only one small clinical study has attempted to relate the treatment outcome to intraradicular bacterial status ($p = 0.025$, Fisher's exact test): the strength of the association was not great, with a wide confidence interval (odds ratio = 6.8; 95% CI: 1.5 to 32). The extent to which current canal sampling techniques accurately reflect the bacterial status of the canal space must also be taken into account. False positive and negative cultures may adversely affect the performance of MRS. These conditions emphasize how potentially error-prone MRS can be. As currently practiced, intracanal sampling techniques suffer from deficiencies that limit their predictive value. This article in no way questions the role of intracanal bacteria in causing apical periodontitis, nor the central role of bacterial control in endodontic treatment. Rather, it emphasizes the need for more detailed clinical studies of bacterial status and healing, as well as refinement of techniques for microbial sampling of canals. *(J Endod 2007;33: 220–225)*

Key Words

Evidence-based, microbiology, performance, surrogate endpoint

From the Endodontic Unit, School of Dental Science, The University of Melbourne, North Melbourne, Victoria, Australia.

Address requests for reprints to Dr. Chankhrit Sathorn, Endodontic Unit, Department of Restorative Dentistry, School of Dental Science, The University of Melbourne, 720 Swanston Street, North Melbourne, Victoria 3010, Australia. E-mail address: chankhrit@gmail.com.

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It has been well established that apical periodontitis is caused by bacteria within the canal space (1, 2). The treatment of apical periodontitis should thus be removal of the cause—that is, bacterial eradication. As a result, current treatment protocols (isolation, canal preparation, antibacterial irrigants, and intracanal medicaments) are directed toward bacterial elimination. Because of this intimate involvement of bacteria and clinical endodontics, the rationales for and development of treatment protocols are all based on data involving microbiological root canal sampling (MRS) (3–8). In other words, MRS is the very foundation of clinical endodontics.

MRS results have been used widely in clinical endodontic research as a predictor (a surrogate endpoint) for clinical outcomes (healing). A surrogate endpoint is a variable that is relatively easily measured and that predicts a distant outcome of a therapeutic intervention, but which is not in itself a direct measure of clinical benefit (9). A major important feature of surrogate endpoints is that they can dramatically reduce the sample size, duration, cost, and thus difficulties of clinical studies. Its shortcoming, however, is that the surrogate endpoint may not closely reflect the treatment target; in other words it may not be sensitive enough to predict treatment outcome effectively.

In this article we address the question of the extent to which positive or negative cultures at the time of obturation adequately predict treatment outcome—that is, healing of apical periodontitis.

Microbiological Root Canal Sampling (MRS)

MRS entails several technique-sensitive steps (10). It is a complicated procedure performed in an extremely challenging environment. The oral cavity is naturally full of bacteria, which can easily contaminate a sample. Based on Möller's work, to perform MRS properly several steps must be followed: (1) The operative field needs to be prepared by scaling of hard and soft deposits and be effectively isolated with a rubber dam. (2) The operative field is sterilized with 30% hydrogen peroxide followed by 5% tincture of iodine. (3) Sterility of the operative field is monitored by sampling tooth surfaces with a charcoal-impregnated pellet. This pellet will be cultured and, if it is positive to bacteria, any microbiological data obtained from that particular tooth must be discarded because of the risk of contamination. (4) The root canal is filled with sampling fluid; a sample is taken with charcoal-impregnated points that are transferred to a transport medium. (5) The medium is then serially diluted and inoculated into an appropriate nutrient broth and/or agar plates. These are aerobically and anaerobically incubated for a period of time long enough to allow even slowly growing species to form colonies. (6) Results are microbiologically analyzed using growth/no growth determination or identification of isolated microorganisms based on colony morphology, micromorphology, and both physical and biochemical tests (11).

Basically, MRS is a passive sample of the main root canal space, which does not include inaccessible areas—such as accessory canals, fins, and dentinal tubules—nor adherent biofilms. Anaerobic sampling and cultivation techniques are indispensable to analyses of MRS and they are far from straightforward. Special medium supplemented with agar is generally required to prevent oxygen diffusion so that toxic intermediates of oxygen do not accumulate and interfere with viability of anaerobic bacteria (12). Moreover, to obtain growth from potentially positive samples, the incubation time must not be less than about 2 weeks, which, from a microbiological research perspective, is costly and time-consuming (10).

Culture-based identification methods are often unable to reproduce growth conditions required by fastidious bacteria that have stringent environmental and nutritional require-

ments. In this respect, many bacterial species are difficult or impossible to culture and evidence is now emerging that there are species involved in endodontic infections that are uncultivable (13, 14). Because of the limitations of culture-based identification methods, a relatively new technique in microbiology has been developed to identify microorganisms without culturing. Molecular-based identification methods [such as polymerase chain reaction (PCR)] are designed to detect microbial DNA rather than living microorganisms. A limitation of PCR is that it cannot distinguish between DNA from viable or dead cells and it is thus unclear whether the results from this method truly represent the authentic living endodontic flora or, rather, a historical record of organisms that have entered but not survived in the root canal (15). It is important to realize that clinical samples for PCR analysis are acquired by the same method as for culturing (i.e., soaking up sampling fluid from the root canal with paper points) and are thus equally susceptible to contamination and to false positive and/or negative results.

The focus of this review is, however, on MRS and culture-based identification methods, because molecular-based methods are still under continuous improvement and long-term endodontic clinical outcome studies using molecular methods are unavailable at this stage.

Data Reported from MRS

Some studies report MRS results as bacterial counts and/or bacterial species; however, the clinical significance of all bacteria in endodontics is not fully known. Thus, a link between bacterial load/bacterial species and clinical outcomes has never been firmly established. The majority of the endodontic studies that measure therapeutic efficacy of medicaments or other treatment protocols have reported MRS results simply as positive or negative cultures (3–8). Positive or negative culture results have been related to clinical outcomes (healing), either by correlation within the same study (16) or interpreted as a predictor for healing (6–8). The effect of intraradicular bacterial status on treatment outcome was the focus of previous investigations (17–21); however, these older studies were not considered in this review on the grounds that inadequate microbiological techniques were used (no anaerobic culturing).

A complex relationship is present between MRS results and clinical outcomes. First, a majority of cases (68%) healed even with a positive culture at time of obturation, although the frequency of healing was lower than that in teeth with a negative culture (94%) (16). Second, although calcium hydroxide medication tends to reduce the number of positive cultures, this reduction does not result in a higher incidence of healing relative to root canal treated teeth without calcium hydroxide medication (22, 23). Third, in culture studies of endodontically treated teeth with and without persistent lesions, a high proportion of healed cases still had positive cultures, whereas a high proportion of teeth with persistent lesions had negative cultures (24). Therefore, MRS results and clinical outcomes do not show a simple all-or-none relationship. Substantial numbers of canals with positive culture can heal and a number of unhealed cases yield negative cultures. Two questions arise from these observations: (1) How strong is the association between MRS results and outcome? and (2) Does MRS adequately reflect the true bacterial status of the canal space?

How Strong is the Association Between MRS Results (Positive vs. Negative Cultures) and Clinical Treatment Outcome (Healing)?

Persistent Disease and Culture Studies in Initial Treatment

Essentially 100% of teeth with periapical lesions have positive cultures before treatment begins (22, 25, 26). There is no question of the relationship between intracanal bacteria and periapical disease (1, 2). However, when looking at treatment-related issues, and specifically the results of MRS and clinical healing, a more complex picture emerges.

TABLE 1. Raw data of Sjögren et al.[59] investigating the effect of intraradicular bacterial status (as determined by MRS and culturing) on treatment outcome in initial treatment cases

Parameter	Not Healed (%)	Healed (%)	Total
Positive to bacteria cultures	7 (32%)	15 (68%)	22
Negative to bacteria cultures	2 (6%)	29 (94%)	31
Total	9	44	53

No significant difference exists in outcomes for single- and multiple-visit endodontics (23). The clinical difference between single- and multiple-visit endodontics is that root canals are medicated with calcium hydroxide (or other intracanal medicaments) in multiple-visit endodontics. Calcium hydroxide intracanal dressing has been associated with enhanced bacterial elimination, as demonstrated by a reduction in the number of positive root canal cultures (22). Consequently, multiple-visit endodontics should have a higher chance of achieving negative culture status before obturation. However, this higher chance does not equate to higher healing rates, as documented by three randomized controlled clinical trials (27–29) and a meta-analysis of such trials (23).

Only one study with an acceptable microbiological sampling technique has directly associated the clinical outcome (healing) of teeth with positive vs. negative cultures at the time of obturation (16). In this study of 53 single-rooted teeth, all canals were prepared and obturated in a single visit (no intracanal medication used). Culture status was determined from post-instrumentation samples taken from each canal. All samples were meticulously processed under strict anaerobic techniques. Twenty-two cases yielded positive cultures, whereas 31 cases were negative cultures; these cases were followed separately for healing (Table 1). Patients were recalled yearly for clinical and radiographic examination. Radiographs were taken using a standardized technique to obtain optimal diagnostic quality. The cases were followed for 5 years if complete healing had not occurred earlier. Healing was strictly determined using Strindberg's criteria (30). Ninety-four percent of canals with negative cultures healed, whereas 68% of canals with positive cultures healed, and culture status was significantly associated with clinical outcome ($p = 0.025$, Fisher's exact test).

Earlier studies that evaluated the association of intraradicular bacterial status and treatment outcome (17–21) are of limited value because inadequate microbiological techniques were used (no anaerobic culturing). These studies reported small differences in outcome of 10 to 15%. A recent article reported a similar conclusion to Sjögren et al. (16); that is, negative cultures were significantly associated ($p < 0.01$) with successful clinical outcomes (31). In this study of 30 cases, however, bacterial status before completion of cleaning and shaping procedures was used to calculate this association rather than sampling immediately before obturation. Therefore, the results were not indicative of final bacterial status before obturation and cannot be interpreted in the same fashion as Sjögren et al. (16).

Considering bacterial status before obturation as a risk factor for disease (in this case persistent apical periodontitis), the concept of risk difference and odds ratio can be used, and analysis of the study of Sjögren et al. (16) can be carried one step further by quantifying the strength of the association (Table 1).

Risk difference is defined as the risk in the experimental group minus risk in the control group. For the purpose of this study it is given as the difference in healing rates between teeth with negative and positive cultures. Risk difference is a measure of the impact of the treatment on the number of events (healing) because it takes into account the prevalence of the event (i.e., how common it is). This is in contrast to odds ratio, which is a measure of the association between treatment and

TABLE 2. Raw data of Molander et al.[24] investigating the effect of intraradicular bacterial status (as determined by MRS and culturing) on treatment outcome in retreatment cases

Parameter	Not Healed (%)	Healed (%)	Total
Positive to bacteria cultures	68 (68%)	9 (45%)	77
Negative to bacteria cultures	32 (32%)	11 (55%)	43
Total	100	20	120

outcome, but does not give an indication of the impact of the intervention; that is, the same odds ratio can give a different impact depending on how common the event is (32).

Teeth with negative cultures had a reduced risk of persistent disease of 25.4 percentage points (risk difference = 25.4%; 95% CI: 4.6–36.3%) (33). The odds for apical lesion of teeth with negative cultures to heal completely after treatment are 6.8 times that of teeth with positive cultures (odds ratio = 6.8; 95% CI: 1.4–32). An odds ratio of this magnitude may seem to be a very strong association; however, from an epidemiological perspective this number may not be particularly high (34). Relatively, intraradicular bacterial status may not be as strong a predictor of clinical outcomes as historically perceived. In addition, the wide confidence interval of risk difference (4.6–36.3%) and odds ratio (1.4–32) reflects a low precision of the study; that is, there is a 95% chance that a true value of risk difference in a population could be anywhere between 4.6 and 36.3% and the true odds ratio could be any value between 1.4 (almost no association) and 32 (strong association).

Relying on one small study as the basis for treatment recommendations is not very prudent. A useful analogy is the series of clinical studies comparing the effects of quality of obturation and restoration (apical vs. coronal seal) on healing. In the first such study Ray and Trope (35) concluded that the quality of coronal restoration was significantly more important than the quality of endodontic filling to treatment outcome ($p < 0.001$, χ^2 test; odds ratio = 2.6; 95% CI; 1.8–3.9). However, a succeeding article (36) reported the completely opposite result. When five studies investigating the same issue (35–39) were identified and were statistically combined, a different picture emerged. The highly significant result in Ray and Trope (35) became nonsignificant ($p = 0.55$) with an odds ratio of 0.8 (95% CI; 0.3–1.8; meta-analysis data using random effect method, RevMan Version 4.2.7). This emphasizes the point that one study is not sufficient and that clinical confirmation is needed.

If intraradicular bacterial status at the time of obturation were the only factor influencing clinical outcomes, bacterial status should be a perfect outcome predictor. However, the idea of "single factor produces single effect" is probably too simplistic and does not fit the complex relationship between host and disease. The host response and/or quality of coronal restoration could also influence endodontic clinical outcomes (35–40). It is unrealistic to expect that a culture taken at a single time point immediately before obturation will be a perfect predictor of outcome.

Persistent Disease and Culture Studies in Endodontically Treated Teeth

MRS has revealed that 32–56% of endodontically treated teeth with persistent apical lesions (failed cases) were negative to bacterial cultures (24, 41). Causes other than intraradicular infections may be responsible for persistent lesions, such as true cyst, extraradicular infection, foreign body reaction, and scar tissue (42), which cannot be managed by orthograde endodontic treatment. However, given the high healing incidence (>90%) of orthograde endodontic treatment (43), causes of persistent lesions other than intraradicular infections should not account for >10% of cases. Taking this into consideration, the percentage of failed cases negative to bacterial cultures is rather higher than might be predicted (24, 41).

Only one study directly compared the culture status of endodontically treated teeth with and without persistent apical lesions (24). In that study, 100 teeth with persistent lesions were cultured after removal of the root canal filling and compared with 20 teeth without a lesion (retreated for restorative reasons). All teeth had been root treated at least 4 years before the commencement of retreatment to ensure that all apical lesions can be considered "failure" (30). An anaerobic sampling technique as laid out by Möller (10) was strictly followed. In 99 cases, root filling material was successfully removed only by mechanical means to make certain that the bacterial milieu was minimally disrupted. However, in 21 cases chloroform was required to soften the gutta percha. The study showed that 45% of "healed" teeth had positive cultures and 32% of "diseased" teeth showed negative culture. A subsequent study using molecular techniques also substantiated that residual bacteria can be detected in a high proportion (77%) even of successful cases (44).

Even though this result is counterintuitive, the raw data from Molander et al. (24) show that the results of MRS in endodontically treated teeth have no significant association with clinical outcomes ($p = 0.09$, χ^2 test with Yates correction; OR = 2.6 95% CI: 1–6.8) (see Table 2). However, calculations were based on only a small number of endodontically treated teeth without lesions ($n = 20$). Moreover, the sample size of the two groups in the comparison was considerably different (100 vs. 20), making statistics less efficient; the inference power was lower as a result. Finally, the study was cross-sectional in design, which has a limited ability to indicate a causal relationship.

In summary, the association between MRS results and healing is based solely on one small study ($n = 53$), resulting in a wide confidence interval for risk difference and odds ratio (16). More clinical studies are needed. A relatively high proportion of failed cases are found to have negative cultures and a high proportion of successful cases to have positive cultures. This evidence strongly suggests that root canal bacterial status as determined by MRS and culturing has limited value in predicting clinical success (healing). Given the unquestioned relationship between intracanal bacteria and apical periodontitis (1, 2, 25) and the low frequency of nonbacterial factors in persistent disease (42), the question then arises: To what extent do current canal sampling techniques accurately reflect the bacterial status of the canal space?

Does MRS Result Adequately Reflect the True Bacterial Status of the Canal Space?

Theoretical Framework

To determine whether a test measures what it claims, the "truth" or "gold standard" needs to be known, against which test results are compared. Two × two tables can then be constructed (Table 3). Five measures can be derived from this table and used to evaluate the test

TABLE 3. Two × two table for evaluation of the test

	The Truth or "Gold Standard"		Total
	+	−	
Test results			
+	A (true positive)	B (false positive)	A + B
−	C (false negative)	D (true negative)	C + D
Total	A + C	B + D	A + B + C + D

TABLE 4. Derived data from two × two table and their meanings

Evaluating Point	What Does it Mean? (59)
Sensitivity (%), A/(A + C)	How good is this test at detecting canals with bacteria present?
Specificity (%), D/(B + D)	How good is this test at correctly excluding canals without bacteria?
Positive predictive value (%), A/(A + B)	If the test is positive, what is the chance that the canal actually has bacteria present?
Negative predictive value (%), D/(C + D)	If the test is negative, what is the chance that the canal actually does not have bacteria?
Accuracy (%), (A + D)/(A + B + C + D)	The correct result was given by what proportion of all tests?

(Table 4). Sensitivity and specificity tell about the test in general, whereas the predictive value tells about what a particular test results mean for the particular tooth/canal. For that reason sensitivity and specificity are more appropriate than others to measure performance of the test.

Performance of Microbiological Root Canal Sampling

In terms of evaluating the accuracy of root canal sampling, the truth is not known unless all tested teeth can be assessed by an independent measurement that is known to be reliable (the "gold standard"). One article directly demonstrated that negative root canal cultures did not necessarily indicate sterility of the root canal (45). In this study, 20 infected human teeth were extracted. The outer tooth surface was sterilized by means of ultraviolet light. Sterilization of the outer tooth surface was controlled and examined by streaking on agar plates. If sterilization was not successful, data from that particular tooth were discarded. Canals were then accessed and prepared extraorally. Root canal samples were taken using paper points and were cultured aerobically and anaerobically. All teeth were finely crushed and the tooth powder was cultured. Viable bacteria were still found in seven teeth despite three consecutive negative cultures of MRS after complete canal preparation and medication.

It is ethically unfeasible to extract human teeth simply to establish a gold standard. To circumvent this problem, root canals can be sampled at the subsequent session after canals had been left empty or filled with nutrient broth, allowing previously irretrievable bacteria to repopulate the root canal system. This technique has been used and called "gold standard" in three articles that systematically assessed the accuracy of MRS after canal medication (46–48). The results are shown in Table 5.

Sample size in all three studies was relatively small, resulting in wide confidence intervals (Table 5, sensitivity column) and low precision. One commonality among the three studies was that sensitivity of MRS was strongly influenced by the type of intracanal medication. Calcium hydroxide especially affected MRS sensitivity a great deal. Sensitivity was reduced to only 33% when canals had been medicated with calcium hydroxide (46). That means that a relatively high number of canals were false negative (i.e., negative cultures at initial sampling but positive at "gold standard" sampling). It seems to be a scientific dilemma to evaluate the efficacy of intracanal medication using MRS because the test itself can be heavily influenced by the tested materials.

These calculations substantially depend on determining false positive and false negative results, established from the number of culture reversals in MRS. A culture reversal is defined for the purpose of this analysis as the number of negative cultures at initial sampling, which subsequently turn positive at the "gold standard" sampling, and vice versa. In other words, the higher the number of culture reversals, the lower the accuracy of the MRS.

Culture reversal means there is a change in bacterial status between the first (test results) and second visit (gold standard) where canals have been left empty or filled with nutrient medium between visits. It was previously demonstrated that bacteria can regrow in empty canals. Thirty-six of 150 canals (24%) (49) and four of 12 canals (33%) (31) showed a negative culture at the first visit but were positive at the second visit 7 days later when canals were left empty. On the contrary, bacteria also appear to die off in empty canals. Three of 15 canals (20%) with positive cultures at the first appointment (after canal preparation and irrigation) were negative at the next appointment 2 to 4 days later (4, 5). Regardless of the direction, culture reversal contributes substantially to the inaccuracy of MRS.

Why is Performance of MRS Not Particularly High?

Several possible factors play a part in culture reversal or low performance of MRS.

False Negative

It is possible that bacteria may be in the root canals from the very beginning but located in inaccessible areas for MRS, such as ramifications and dentinal tubules (50). They can repopulate root canals after the first MRS shows a negative result. In addition, a carryover effect of antibacterial irrigants or medicaments can inhibit bacterial growth during the cultivation process, provided that neutralizing procedures were not available or were not conducted properly.

False Positive

MRS is a technique-sensitive procedure performed in a hostile setting. Despite every precaution being taken as Möller (10) suggested, the field of operation for endodontic procedures is not entirely sterile nor completely immune from saliva leakage and recontamination.

In addition to these false positive or negative situations, the bacterial status of the canal may actually change between appointments for

TABLE 5. Summary of diagnostic accuracy of MRS

Study	Medication	*n* (teeth)	Sensitivity (%) A/(A + C) (95% CI: % to %)	Specificity (%) D/(B + D)	Positive Predictive Value (%) A/(A + B)	Negative Predictive Value (%) D/(C + D)	Accuracy (%) (A + D)/(A + B + C + D)
Reit and Dahlén (1988) (46)	Calcium hydroxide	35	33 (7.5 to 70)	81	38	78	69
Molander et al. (1990) (47)	Clindamycin	24	50 (12 to 88)	94	75	85	83
Reit et al. (1999) (48)	IKI	50	68 (45 to 86)	75	69	75	72

"incidental" reasons unrelated to the study itself. When intraradicular bacterial status has truly changed between initial and gold standard sampling, for the purpose of this analysis, this situation is labeled "incidental."

Incidental False Negative

Bacteria may die off because of an inappropriate or nutrient-deprived root canal environment. Another possibility is that if bacteria are low in number at the initial sampling, at the second visit they might not be retrieved simply by chance. In these conditions, even though MRS has accurately detected what it is meant to detect (i.e., the actual change in bacterial status), the results will be shown as culture reversal, which will unfavorably affect the measurement of performance of MRS. In other words, MRS has measured bacterial status correctly, but the results will be shown as incorrect and it is impossible to determine whether it is actually incorrect.

Incidental False Positive

Bacteria can reenter the root canal system between visits through coronal leakage of the temporary restoration and/or marginal deficiency (51), cracks, and exposed dentinal tubules (52). Anachoresis is possibly another potential explanation, although generally regarded as a remote possibility (53–56). Again, MRS may measure bacterial status correctly but the result will be interpreted as a reversal.

Four potential conditions that may adversely affect performance of MRS have been outlined: false negative, false positive, incidental false negative, and incidental false positive. These conditions emphasize how error-prone MRS and the measurement of the performance of MRS can be.

The problem with performance of MRS appears to be one of sampling rather than the bacterial identification process. Given the possibility of biofilms and bacteria in inaccessible areas such as ramifications and dentinal tubules, passive sampling of the main canals is not likely to detect all bacteria. Ørstavik et al (57) attempted to solve the problem by additional filing of the canal wall, but this will not reach inaccessible areas. Low-level ultrasonic agitation has been used in microbiological research to segregate clumped bacteria without injuring the cells (58), and a similar approach could probably be applied in root canal sampling. Its ability to dislodge bacteria from inaccessible locations especially deep within dentinal tubules is unknown.

Conclusions

Bacterial culturing is not an end in itself—its main purpose clinically is to serve as a predictor of healing. To date only one small study (53 cases) (16) has documented an association between culture status at the time of obturation and healing. The only study of persistent lesions and culture status of endodontically treated teeth (24) did not demonstrate a significant association. More clinical studies associating intraradicular bacterial status to healing rates are required. Culturing canals after the use of an intracanal medicament (especially calcium hydroxide) may largely demonstrate the carryover effect of residual medicament rather than elimination of bacteria from the canal space, despite efforts to minimize the carryover. Thus, as currently practiced, intracanal sampling techniques suffer from deficiencies that limit their predictive value. This conclusion in no way questions the role of intracanal bacteria in causing apical periodontitis, nor the central role of bacterial control in endodontic treatment. Rather, it emphasizes the need for more detailed clinical studies of bacterial status and healing, as well as refinement of techniques for microbial sampling of canals.

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