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BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte AYOUB RASHTCHIAN and DAVID M. SCHUSTER

Appeal 2019-005017
Application 10/633,629
Technology Center 1600

Before DEBORAH KATZ, JOHN G. NEW, and RYAN H. FLAX, *Administrative Patent Judges*.

KATZ, *Administrative Patent Judge*.

DECISION ON APPEAL

Appellant[1] seeks our review,[2] under 35 U.S.C. § 134(a), of the Examiner's decision to reject claims 43–49, 52, 53, and 56–64. We have jurisdiction under 35 U.S.C. § 6(b). We AFFIRM.

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[1] We use the word "Appellant" as defined in 37 C.F.R. § 1.42. Appellant identifies the real party-in-interest as Qiagen Beverly, Inc. (Appeal Br. 1.)
[2] We consider the Final Office Action issued December 28, 2017 ("Final Act."), the Appeal Brief filed July 27, 2018 ("Appeal Br."), the Examiner's Answer issued on April 12, 2019 ("Ans."), the Reply Brief filed June 12,

The Examiner rejects claims 43–47, 52, 56–61, 63, and 64 under 35 U.S.C. § 103(a) over Li,[3] McMillan,[4] Lopez Garcia,[5] Durmowicz,[6] and Kyle.[7] (Ans. 4–6.)

The Examiner also rejects claims 43–48, 52, and 56–64 under 35 U.S.C. § 103(a) over Li, McMillan, Lopez Garcia, Durmowicz, Kyle, and Blaschke.[8] (*See* Ans. 6.)

The Examiner entered a new ground of rejection for claims 49 and 53 under 35 U.S.C. § 103 over Li, McMillan, Lopez Garcia, Durmowicz, Kyle, and Wierenga.[9] (Ans. 7–8.)[10]

2019 ("Reply Br.") and the oral argument held on May 6, 2020, in reaching our decision.

[3] Li and Wang, "Application of real-time polymerase chain reaction for the quantitation of interleukin-I~ mRNA upregulation in brain ischemic tolerance," *Brain Research Protocols*, 5:211-217 (2000).

[4] McMillan et al., "Methods for Quantitative Analysis of Nucleic Acid Amplification Reaction," U.S. Patent No. 6,783,934B1, issued August 31, 2004.

[5] Lopez Garcia et al., "Use of Flow Injection Flame Atomic Absorption Spectrometry–for Slurry Atomization. Determination of Copper, Manganese, Chromium *and Zinc in Iron Oxide Pigments*, Analyst 116:517–20 (1991).

[6] Durmowicz, et al., "Detection of Neisseria Gonorrhoeae by Amplification and Detection of Its Nucleic Acid," US Patent No. 5,962,273, issued October 5, 1999.

[7] Kyle, "Arachidonic Acid and Methods for the Production and Use Thereof," US Patent No. 5,658,767, issued August 19, 1997.

[8] Blaschke et al., "Rapid quantitation of proinflammatory and chemoattractant cytokine expression in small tissue samples and monocyte-derived dendritic cells: validation of a new real-time RT-PCR technology," *J. Immunol. Methods*, 246:79–90 (2000).

[9] Wierenga, US Patent 5,968,889, issued October 19, 1999.

[10] In the Answer, the Examiner stated that the instant method is not enabled with respect to using antifoam O-30 because antifoam O-30 was

Appellant's Specification is directed to methods of real-time nucleic acid amplification that use anti-foam reagents. (Spec. 1:12–15.) Appellant explains that analysis of real-time polymerase chain reaction ("RT-PCR") involves quantifying how much template the polymerase produces by measuring a fluorescent signal incorporated into the template with each amplification cycle. (*See id.* at 3:9–16.) According to Appellant, determining baseline fluorescence is a major problem in the analysis of RT-PCR data because the baseline varies for each reaction, increasing and decreasing without relation to amplification of the template. (*See id.* at 3:29–30.) Appellant's Specification states that "[t]hese problems are often exacerbated by bubbles in the reaction that interfere with optical measurements" and are likely caused by non-ionic polymeric detergents, which are, nevertheless, a necessary component of the amplification reaction. (*Id.* at 3:33–4:2.)

The Specification states that the anti-foam reagents decrease variation in the fluorescence background in enzyme assays, "by reducing optical interference from bubbles that would otherwise change the fluorescent signal in a manner that is not dependent on the concentration of the desired nucleic acid product." (Spec. 5:9–15.)

Appellant's claim 43 recites:

> A method for detecting a target nucleic acid in a sample, comprising the steps of amplifying the target nucleic acid using a real-time quantitative polymerase chain reaction and detecting

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discontinued. (*See* Ans. 3.) The Examiner did not formalize a rejection based on 35 U.S.C. § 112, first paragraph. Thus, we do not consider the claims to be rejected on that basis and do not consider Appellant's arguments in response to such a rejection. (*See* Reply Br. 17–19.)

> the product of said polymerase chain reaction by optical detection,
>
> wherein said real-time quantitative polymerase chain reaction is carried out in the presence of:
>
> a thermostable DNA polymerase suitable for temperature cycling between high and low temperatures;
>
> a detergent;
>
> and at least one anti-foam reagent at a concentration of less than 0.01%, wherein said anti-foam reagent is selected from the group consisting of 1520-US, AF, FG-10, O-30, SE-15, and Antifoam B.

(Appeal Br. 77.) Appellant's claim 52 is also independent and recites a composition for quantifying a target nucleic acid by real-time PCR, including, *inter alia*, "(e) at least one anti foam reagent at a concentration of less than 0.01%, wherein said anti-foam reagent is selected from the group consisting of 1520-US, AF, FG-10, O-30, SE-15, and Antifoam B." (*Id.* at 78.)

Examiner's Rejection of claims 43–47, 52, 56–61, 63, and 64 under 35 U.S.C. § 103(a) over Li, McMillan, Lopez Garcia, Durmowicz, and Kyle

We begin our review of the Examiner's rejection and Appellant's arguments by focusing on independent claim 43. The Examiner cites Li for its teaching of TaqMan hot-start RT-PCR for quantitation of mRNA. (*See* Ans. 4, citing Li abstract.) Li teaches including the detergent Tween 20 in the "TaqMan PCR core reagents." (*See* Li 212; *see* Ans. 4.) As the Examiner notes, Li does not teach including an anti-foam agent in the reactions. (*See* Ans. 4.)

The Examiner cites McMillan for its teaching that air bubbles can have a negative effect on optical detection in amplification reactions such as RT-PCR. (*See* Ans. 4, citing McMillan 3:57–4:11 and 35:54–57.) The

Examiner further cites Lopez Garcia for its teaching that abundant foam from surfactant can create reproducibility problems because of bubbles trapped in the sample loop in a procedure for determining copper, manganese, chromium, and zinc in slurries of commercial iron oxide pigment using spectrometry. (*See* Ans. 4, citing Lopez Garcia 518.) Lopez Garcia teaches further that "this problem could be overcome by adding several drops of antifoaming agent . . . to the slurry," but that because detergent decreased the absorption signal for copper, the surfactant was eliminated instead. (Lopez Garcia 518.)

The Examiner cites Durmowicz for its teaching to include anti-foam reagents in a polymerase reaction for optical detection of an amplified nucleic acid. (*See* Ans. 4, citing Durmowicz abstract, 26:20.) Durmowicz teaches strand displacement amplification ("SDA") assays, which are distinct from PCR reactions and use a different polymerase. (*See* Durmowicz abstract.)

The Examiner cites Kyle for its teaching of the use of silicone-based anti-foaming agent 1520-US in a fermentation reaction. (*See* Ans. 5, citing Kyle 11:53–54.)

As the Examiner finds, the prior art teaches detecting target nucleic acid in a sample using RT- PCR and detecting the product of the reaction by optical detection. (*See* Li abstract.) The prior art also teaches that this reaction includes a detergent, but that bubbles can have a negative effect on optical detection of the amplification products of RT-PCR. (*See* Li 212, McMillan 3:57–4:11 and 35:54–57.) Although the Examiner does not cite to the use of anti-foam reagent in RT-PCR specifically, anti-foam was known to have been used with a different polymerase. (*See* Durmowicz abstract,

26:20.) The prior art also shows that at least one of the anti-foams recited in claim 43 was known in the art. (*See* Kyle 11:53–54.)

The Examiner determines that it would have been obvious to one of ordinary skill in the art to modify the method of Li by further including an anti-foam agent in a concentration of up to 0.015% in the master mix to improve optical detection in real-time PCR. (*See* Ans. 5.) The Examiner notes that although the prior art does not specifically teach the range of 0.01% or less anti-foam reagent, one of ordinary skill would have found it obvious, requiring only routine experimentation, to vary the anti-foam concentration with the intent of optimizing the PCR reaction. (*See id.*; *see also id.* at 9–10, 35–36.)

Appellant's Evidence of Unexpected Results

We turn to Appellant's evidence of unexpected results in order to review the substantive record as a whole. *See In re Packard*, 751 F.3d 1307, 1312 (Fed. Cir. 2014) ("The 'prima facie case' determination is a purely procedural device that operates at the examiner level to clarify how the interaction process proceeds. Thereafter any final rejection by the examiner, and any review of the rejection, whether by the Board or through appeal to the courts, turns on the substantive question of the merits of the rejection."); *see also In re Rinehart*, 531 F.2d 1048, 1052 (CCPA 1976) ("When prima facie obviousness is established and evidence is submitted in rebuttal, the decision-maker must start over. . . . An earlier decision should not, as it was here, be considered as set in concrete, and applicant's rebuttal evidence then be evaluated only on its knockdown ability. . . . [A] final finding of obviousness may of course be reached, but such finding will rest upon

evaluation of all facts in evidence, uninfluenced by any earlier conclusion reached . . . .")

Appellant argues that the method of the rejected claims improves the efficiency, accuracy, and precision of the RT-PCR reaction when compared to RT-PCR without an anti-foam reagent. (*See* Appeal Br. 46–55.) Appellant asserts that improving optical detection and improving RT-PCR efficiency are distinct outcomes and that the claimed method unexpectedly accomplishes the later as well. (*See id.* at 49.)

Appellant cites to Examples I, II, and III of the Specification to show that the *efficiency* of RT-PCR with anti-foam reagents is higher than in samples without anti-foam reagent and points to Examples IV and VI[11] to show the RT-PCR with anti-foam reagent has improved *precision* and *accuracy*. (*See* Appeal Br. 49–50.) Appellant refers to Example V, as well. (*See id.* at 52–53.)

Appellant also cites to the Declaration of inventor David Schuster, submitted under 37 C.F.R. § 1.132 on December 4, 2017 ("Schuster Decl.") to support these arguments. (*See* Appeal Br. 53.) Mr. Schuster states that he reviewed the prior art cited by the Examiner in the Office Action mailed May 11, 2016. (*See* Schuster Decl. ¶ 9.) The rejections in that Office Action do not cite McMillan or Durmowicz. Accordingly, Mr. Schuster's

[11] Appellant does not provide specific argument about Example VI. The description of Example VI in Appellant's Specification appears to be a copy of the description of Example IV. (*Compare* Spec. 27–28 *with* Spec. 28–30.) Thus, even though Appellant refers to Example VI in the Appeal Brief, we do not review the results it presents separately from those of Example IV.

declaration does not address the current rejection of Appellant's claims. We review Mr. Schuster's testimony to the extent it is relevant to the current rejection.

The results of many of the experiments in Appellant's Specification are reported as "Ct" values. Appellant explains that "Ct" is the intersection between an amplification curve and a threshold line and is a relative measure of the concentration of target in the PCR reaction. (*See* Appeal Br. 50.) Appellant reports that many factors impact the absolute value of Ct besides the concentration of the target so that "Ct values from PCR reactions run under different conditions or with different reagents cannot be compared directly." (*Id.*) It is not clear what impact this statement has on the comparisons of Ct values from different PCR performed under different conditions that are presented in the examples of Appellant's Specification.

Appellant argues that Example I shows "the effect of antifoam reagent 1520-US at concentrations of at least 0.01%, 0.001% and 0.0001% *increase the PCR efficiency* from 91.1% (no antifoam reagent; 0%) to 96.5%, 98.5% and 97.7%, respectively." (Appeal Br. 50.) The results of the experiments in Example I are reported in Table 1 of Appellant's Specification, which is reproduced below.

	Threshold Cycle (Ct) Results Concentration of DOW 1520-US				
DNA Target Amount (copies)	0%	0.1%	0.01%	0.001%	0.0001%
1×10^2	35.19	33.667	35.403	34.723	35.604
	35.43	33.97	33.979	33.363	33.907
	35.27	34.195	33.743	34.012	33.409
1×10^4	27.8	26.557	26.937	27.051	27.11
	27.93	27.079	26.867	27.131	27.202
	28.02	27.208	27.275	27.183	27.489
1×10^6	21.04	20.207	20.495	20.501	20.753
	20.94	19.916	20.302	20.613	20.575
	21.25	20.419	20.707	20.613	20.661
1×10^8	13.87	13.736	13.716	13.76	14.003
	13.96	13.642	13.816	13.804	13.931
	13.8	13.708	13.935	13.934	14.018
Standard Curve Slope	-3.555	-3.376	-3.409	-3.357	-3.379
Correlation Coefficient	-1.000	-0.9995	-0.9984	-0.9992	-0.9979
PCR efficiency	91.1%	97.8%	96.5%	98.5%	97.7%

Table 1. Effect of Dow 1520-US anti-foam on Taqman real-time quantitative PCR. Summary of Ct values for Taqman PCRs containing varying amounts of anti-foam and DNA target as indicated in the table and corresponding linear regression analysis for the Ct versus log DNA input standard curve.

Table 1 provides "PCR efficiency" determinations for different concentrations of 1520-US, including 0% as a control. We note that no measure of statistical error is reported in Table 1.

The Specification discusses these results, wherein:

> Results demonstrated that the inclusion of anti-foam in PCR does not inhibit the kinetics of DNA amplification or interfere with optical detection of the 5'-nuclease TaqMan assay. Linear regression analysis of the log of DNA copy input versus Ct number indicated that inclusion of anti-foam at all concentrations that were tested, improved PCR efficiency. Concentrations of as low as 0.001%, were sufficient to eliminate bubbles in the reaction cocktail and improve its liquid handling properties. However, anti-foam concentrations of 0.1% or 0.01% imparted a cloudy appearance to the reaction.

> Therefore, optimal concentration of a given anti-foam compound must be determined empirically and is a balance between the cloud point, the efficacy of the compound to eliminate bubbles from surfactants, and any potential adverse effect on DNA amplification.

(Spec. 24:31–25:7.) We agree that Table 1 shows PCR efficiency is higher for each concentration of anti-foam reagent 1520-US than it is for 0%, although it is not clear that this result is statistically significant.

Appellant argues that Table 1 shows that "regardless of the effect on bubbles or the appearance of the reaction mixture in which a cloudy appearance may have a negative effect on optical detection, the *PCR efficiency is still improved* for the claimed concentrations tested." (Appeal Br. 51.) According to Appellant, because reactions with higher concentrations of anti-foam are cloudy, yet still yield increased PCR efficiency, the higher efficiency is not due to reducing bubbles. (*See id.*)

Appellant's argument makes the assumption that cloudiness is caused by bubbles and that a cloudy solution must have significant bubbles. But Appellant does not direct us to evidence of the cause of cloudiness either in the Specification or elsewhere to support this argument. We note that Mr. Schuster testifies that in Exhibit A of his declaration, the addition of anti-foam at low concentrations "yielded a clear solution, lacking any visible precipitate . . . ." (Schuster Decl. ¶ 17.) Thus, clouding may be caused by precipitate instead of bubbles. If the cloudiness of reactions with higher concentrations of anti-foam is due to precipitate and not to bubbles, Appellant's argument that the higher efficiency cannot be due to the lack of bubbles is unsupported.

In general, both Appellant and Mr. Schuster fail to sufficiently explain the interaction between the known optical interference caused by bubbles (*see* McMillan 35:55–56), cloudiness (which could be due to bubbles or precipitate), and the measurements required to determined PCR efficiency. Appellant's Specification states:

> Ideally, the basal fluorescence of a real-time reaction should be invariant from cycle to cycle until the accumulation of PCR product is sufficient to produce signal above background. Surfactants present in PCR and *Taq* DNA polymerase preparations, however, can result in bubbles when reactants are mixed. Failure to clear reactions of bubbles prior to PCR cycling can distort optical signals and skew background fluorescence readings.

(Spec. 27:23–28.) Because Appellant describes Ct measurements as "the intersection between an amplification curve and a threshold line (see, for example, Li, Figure 2)" (Appeal Br. 50), it is not clear to us that bubbles, which can skew background fluorescence readings, would not also impact Ct measurements. It is not clear that bubbles have no impact on Appellant's efficiency calculations, which are based on Ct measurements.

Appellant argues that the increased efficiency of reactions including anti-foam shown in Example I and Table 1 of the Specification are "not identified as being tied to optical detection" and that the Examiner makes an unsupported assumption that optical detection is involved. (Appeal Br. 50–51.) In light of the statement in the Specification and teachings in McMillian that bubbles can distort optical signals and, thus skew background fluorescence readings, we are not persuaded that a link between efficiency and optical detection is completely unsupported. Appellant fails to present sufficient evidence to persuade us that one of ordinary skill in the

art would have considered the increased efficiency reported in Table 1 to have nothing to do with a reduction in bubbles caused by the addition of anti-foam. Appellant does not direct us to evidence of increased efficiency that is based on a parameter independent of optical detection.

Appellant argues further that Examples II and III of the Specification demonstrate different anti-foam reagents at a concentration of less than 0.01% improve PCR efficiency relative to control reactions without anti-foam reagent in both real-time TaqMan and SYBR Green I PCR reactions. (*See* Appeal Br. 51.) The results of Examples II and III are reported in Tables 2 and 3, respectively, which indicate PCR efficiency as apparently determined from Ct values obtained from reactions the included anti-foam reagent and a control reaction without anti-foam reagent.

We agree that Tables 2 and 3 report higher PCR efficiency for reactions including each anti-foam than for the control reaction without anti-foam. We also note that no measure of statistical error was reported for the PCR efficiency determinations in either Table 2 or 3.

Inventor Schuster analyzed the results of Example III, stating that "mean Ct values for amplification of low (e.g., 100) copy samples of at least 1520-US (33.321) and O-30 (33.267) are one cycle lower than control (34.271), providing evidence that anti-foam reagents 1520-US and O-30 improve *efficacy* of low copy nucleic acid analytes in PCR." (Schuster Decl. ¶ 13 (emphasis added).) The numbers that Mr. Schuster reports are a mean of the three Ct values reported for the control, 1520-US, and O-30 at 1 x 10^2 copies of target DNA. Mr. Schuster does not provide a measure of statistical error for these numbers. Furthermore, it is not clear what Mr. Schuster means by "improved efficacy." Mr. Schuster does not discuss the PCR

efficiency values reported in Table 3. Nor does Mr. Schuster state that the "improved efficacy" obtained with 1520-US and Sigma O-30 was unexpected. Mr. Schuster does not state that any of the anti-foams tested in Example III other than 1520-US and Sigma O-30 improve the efficacy of low copy analytes in PCR.

Mr. Schuster's testimony does not persuade us that the results reported in Tables 2 and 3 demonstrate the method Appellant's claim 43 produces unexpected results because Mr. Schuster fails to explain why any improvement in efficacy would have been unexpected or why the increase in efficiency reported was statistically significant. Thus, even if the results with DOW 1520-US were unexpected, the evidence is not commensurate with the full scope of claim 43.

Appellant argues further that Example IV demonstrates anti-foam reagents improve precision and reliability of low copy RT-PCR. (*See* Appeal Br. 52.) Appellant reports that anti-foam 1520-US resulted in an average Ct of 32.62 with a standard deviation of 0.87 compared with the control, which had an average Ct of 33.41 with a standard deviation of 1.29. (*See id.*) Although the Specification and Mr. Schuster support Appellant's argument, we are not persuaded that these results demonstrate anything unexpected about a method of real-time PCR in the presence of 1520-US or any other anti-foam recited in Appellant's claim 43. (*See* Spec. 27:14–16; *see* Schuster Decl. ¶ 14.) Neither the Specification nor Mr. Schuster provides an analysis of the statistical significance of these results. Appellant states that "[r]eporting the standard deviation is the most common measure of precision" (Appeal Br. 50), but it is unclear if the standard deviations reported for Example IV indicates a significant difference in the results for

reactions with and without 1520-US. Neither Appellant nor Mr. Schuster explains why there is a significant and unexpected difference.

Appellant argues that Example V provides a nexus between the claimed method and the reportedly unexpected results because it demonstrates that not any anti-foam at any concentration will improve a PCR reaction. (*See* Appeal Br. 52.) According to Appellant, anti-foam at a concentration of 0.1% inhibited PCR amplification, whereas anti-foam below 0.01% did not. Appellant reports that 1520 US, Antifoam C, Antifoam B, and O-30 were effective. (*See id.* at 53, citing Spec. 28:22–30.) Data supporting these results are not show in the Specification. (*See* Spec. 28:22–28.)

Appellant's declarant, Mr. Schuster, states that the results provided in the Specification are

> evidence that the addition of the claimed antifoam reagents including Dow 1520-US, AF, FG-10, O-30, SE-15, and Antifoam B at concentrations less than 0.01% result in an increased sensitivity of the amplification reactions as evidenced by lower Ct values and higher precision. These results were unexpected and surprising at the time the instant application was filed.

(Schuster Decl. ¶ 15.) We are not persuaded by this statement because Mr. Schuster does not explain why any observed result of adding the anti-foam agents recited would have been unexpected or surprising, given their effects on optical interference. It is not clear what "increased sensitivity" and "higher precision" are and whether they relate to the "PCR Efficiency" reported in Tables 1–3.

Mr. Schuster presents results in Exhibits A and B to his declaration to support his assertion that the method of claim 43 produced results that would have been considered unexpected. (*See* Schuster Decl. ¶¶ 16–19.)
Mr. Schuster reports that Exhibit A shows that each of the anti-foam agents failed to produce a solution with a visible precipitate at concentrations less than 0.01%. (*See* Schuster Decl. ¶ 17.) According to Mr. Schuster, these results would have been unexpected and surprising because they demonstrate that "the claimed antifoam reagents at concentrations less than 0.01% are sufficient to eliminate bubbles without adversely effecting PCR amplification." (Schuster Decl. ¶ 17.)

This statement is not persuasive because Mr. Schuster fails to explain why it would have been unexpected that adding anti-foam would produce a clear solution lacking visible precipitate. Mr. Schuster's determination may reflect his statement that "[i]t was generally believed that anti-foam reagents were incompatible in polymerase reactions" (Schuster Decl. ¶ 12), but this statement is not supported by the prior art cited by the Examiner.
Specifically, the current rejection cites Durmowicz, which teaches including 0.015% anti-foam reagent in a polymerase reaction. (*See* Durmowicz 26:20, Example 14; *see* Final Act. 4.) Mr. Schuster did not consider Durmowicz in his declaration. (*See* Schuster Decl. ¶ 9.)

Mr. Schuster reports that Exhibit B shows that the anti-foam reagents recited in claim 43 "demonstrate identical efficiency and sensitivity across all concentrations tested" and that this is an unexpected and surprising result showing that "antifoam reagents at concentrations less than 0.01% provide reliable and reproducible PCR amplification." (Schuster Decl. ¶ 18.)

Exhibit B provides Ct values for anti-foam reagents at concentrations from 0.1% to 0.0001% with 1 x 10^2 to 1 x 10^8 target copies.

Again, we are not persuaded that the claimed method would produce results unexpected by one of ordinary skill in the art. Mr. Schuster does not explain why one of ordinary skill would have expected less anti-foam reagent to produce unreliable results. Mr. Schuster does not direct us to evidence of the ability of each anti-foam reagent to reduce bubbles at the concentrations tested in Exhibit B or the expectation of those in the art. For example, if it was known that anti-foam reagents can reduce bubbles at 0.0001%, it might not have been unexpected that using anti-foam at this concentration in the claimed method would have produced the similar results as adding anti-foam reagent at 0.01%.

In summary, we are not persuaded by any of the evidence Appellant cites that adding the anti-foam reagents recited in claim 43 produced results that would have been surprising or unexpected to those of ordinary skill in the art. We are not persuaded that higher PCR efficiency would have been unexpected given that it was known in the art that bubbles cause optical interference and PCR efficiency is based on Ct values, which are determined by detecting optical signals.

Appellant's Arguments in Regard to the Prima Facie Case

Appellant raises many arguments against the Examiner's prima facie case for obviousness. (*See, inter alia*, Appeal Br. 10–47.) Many of these arguments address the merits of prior art references individually, but without consideration of their combination with the other cited references. For example, Appellant argues that Li fails to teach anti-foam reagent, McMillan fails to teach *any* component in a reaction mixture that can be used in RT-

PCR, Durmowicz fails to teach a thermostable DNA polymerase or detergent, and Kyle fails to teach a DNA polymerase. (*See id.* at 25.) "Non-obviousness cannot be established by attacking references individually where the rejection is based upon the teachings of a combination of references." *In re Merck & Co.*, 800 F.2d 1091, 1097 (Fed. Cir. 1986).

Appellant attacks the Examiner's finding that McMillan would have provided a motivation to modify the method taught in Li, arguing that McMillan presents a mechanical solution to the problem of bubbles instead of by adding anti-foam reagent. (Appeal Br. 10, 31.) Appellant argues that McMillan fails to teach adding additional components to a reaction mixture. (*See id.* at 20.) We are not persuaded by these arguments because McMillian was not cited for teaching a solution to the problem of bubbles in RT-PCR, but rather to show that those of ordinary skill would have known that bubbles were a problem. (*See* Ans. 4 ("McMillan et al. teach that air bubbles could have a negative effect on optical detection in amplification reactions such as RT-PCR (column 3, lines 57 through column 4, line 11; column 35, lines 54-57).")

Similarly, Appellant argues that Li presents no motivation to modify its own teachings because it fails to cite any problems with reproducibility using optical detection. (*See* Appeal Br. 12.) This argument is unpersuasive because it fails to acknowledge that McMillan teaches the negative effects of air bubbles on optical detection. Whether or not this negative effect was recognized in Li, those of ordinary skill would have been aware of it from at least McMillan.

Appellant argues that Durmowicz fails to teach a protocol combining a detergent, a thermostable polymerase and an anti-foam reagent in RT-PCR

and also fails to specify which anti-foam reagent it used for the reaction described. (*See* Appeal Br. 16.) Durmowicz was cited only to show that anti-foam reagents had been used with a polymerase in the art. (*See* Ans. 4–5 ("Durmowicz et al. teach that antifoam agents in concentration of 0.015% could be successfully used in conjunction with polymerases, i.e., antifoams are not inhibitory (Example 14).").) Durmowicz was not cited for teaching anti-foam reagent in an RT-PCR solution and was not cited for teaching specific types of anti-foam reagents that were known in the art at the time. Thus, Appellant's argument is unpersuasive. Appellant's argument that Durmowicz fails to teach why using an anti-foam reagent is necessary is similarly unpersuasive in light of the Examiner's citation of McMillan. (*See* Appeal Br. 17.)

Appellant argues further that the Examiner inappropriately relied on Durmowicz for a teaching of the concentration of anti-foam reagent recited in claim 43 because the reaction in Durmowicz is different from a PCR reaction. (*See* Appeal Br. 17.) Although the Examiner noted the concentration of anti-foam reagent recited in Durmowicz (0.015%), which is similar to that recited in claim 43 (less than 0.01%), the rejection is based on the ability of one of ordinary skill in the art to determine the claimed concentration based on routine experimentation. (*See* Ans. 5.) Accordingly, Appellant's argument is not persuasive.

In the Reply Brief, Appellant argues that the Examiner failed to establish that the claimed combination of components in a RT-PCR was recognized to be a result-effective variable because it was not recognized as improving amplification efficiency of RT-PCR. (*See* Reply Br. 16.) We are not persuaded by this argument, first, because Appellant has not persuaded

us that anti-foam reagent at added to RT-PCR at less than 0.01% improves amplification efficiency. We are also not persuaded because even if amplification efficiency is improved, one of skill in the art need only have optimized the concentration of anti-foam reagent to reduce bubbles. Claim 43 does not recite a limit on amplification efficiency.

We are similarly not persuaded by Appellant's argument that those of ordinary skill in the art would not have been motivated by Durmowicz to select an anti-foam for use in RT-PCR because Durmowicz teaches using a different polymerase, which is not thermostable and does not require detergent. (*See* Appeal Br. 18.) We are not persuaded by Appellant's argument because Durmowicz was cited only for its teaching that it was known to use anti-foam with a DNA polymerase. We agree with the Examiner that this teaching would have provided the suggestion it might be useful with other DNA polymerases, such as the DNA polymerases used in RT-PCR. We are not persuaded that Durmowicz must have taught the exact conditions of the method of claim 43 for it to contribute to the obviousness of the method. "[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 417 (2007). Adding anti-foam reagent to RT-PCR would not have been beyond the skill of those in the art and Durmowicz relates to polymerases in the same category as the DNA polymerase used in RT-PCR. Thus, we are not persuaded that Durmowicz fails to provide a suggestion to use anti-foam reagent with other DNA polymerases.

Appellant argues that the Examiner improperly relied on Kyle because Kyle relates to fermentation and does not teach a protocol that combines detergent, a thermostable polymerase, and anti-foam reagent suitable for RT-PCR. (*See* Appeal Br. 21.) According to Appellant, the Examiner fails to provide a basis for selecting the anti-foam reagent taught in Kyle. Kyle was cited to demonstrate the knowledge in the art of at least one of the anti-foam reagents recited in claim 43. (*See* Ans. 5 ("Kyle et al. teach the 1520-US as a suitable silicone-based antifoaming agent (column 11, Example 3).") We are not persuaded by Appellant's argument because McMillan and Durmowicz provide a basis for including an anti-foam reagent in RT-PCR. By arguing that Kyle alone fails to teach all of these aspects of the prior art, Appellant fails to consider the entire rejection presented by the Examiner.

Appellant complains that the Examiner cites five separate references to describe "disparate elements" of the claimed method (*see* Appeal Br. 23), but the number of references cited does not indicate non-obviousness. "The criterion . . . is not the number of references, but what they would have meant to a person of ordinary skill in the field of the invention." *In re Gorman*, 933 F.2d 982, 986 (Fed. Cir. 1991). "[F]amiliar items may have obvious uses beyond their primary purposes, and in many cases a person of ordinary skill will be able to fit the teachings of multiple patents together like pieces of a puzzle." *KSR*, 550 U.S. at 420. The Examiner provides a sufficient explanation why one of ordinary skill in the art would have combined the knowledge of RT-PCR components, with the knowledge of the problems caused by bubbles and the knowledge that anti-foams can be used with other DNA polymerases and would have considered it obvious to add a known anti-foam reagent to the RT-PCR solve the problem.

Appellant presents several arguments against the citation of Lopez Garcia, asserting that it is not analogous to the subject matter of the claimed method and should not have been considered. (*See* Appeal Br. 10, 33–44.) A reference is analogous art to the claimed invention if the reference is either in the same field of endeavor or if it is reasonably pertinent to the problem faced by the inventor. *See In re Oetiker*, 977 F.2d 1443, 1447 (Fed. Cir. 1992). For example, prior art teaching sealing arrangements in pressurized carbonated beverages systems have been held to be analogous to claimed cartridges for low pressure liquid chromatography because both allow easy access and variation of the cartridge's contents without destroying its ability to be sealed. *See Sci. Plastic Prod., Inc. v. Biotage AB*, 766 F.3d 1355, 1359 (Fed. Cir. 2014).

Appellant argues that "the problem faced by the inventors was not simply how to eliminate bubbles in RT-PCR for optical detection – it was, in fact, minimizing or reducing baseline fluorescence drift in data analysis of optical measurements in PCR amplification *that use detergents to stabilize thermostable DNA polymerase* (¶ [0009] of the published application)." (Appeal Br. 38.) The cited paragraph states that the problem solved by the method of claim 43 is the interference of bubbles in optical measurements when automating PCR data analysis. (*See* Spec. 3:29–4:2.)

Lopez Garcia teaches using atomic absorption spectrometry to determine the amount of elements in slurries of commercial iron oxide pigments. (*See* Lopez Garcia abstract.) Lopez Garcia teaches that atomic absorption measurement involves introduction of solid samples into a flame and that flow injection ("FI") methods could be a suitable way of avoiding

clogging problems when introducing a suspension into the flame. (Lopez Garcia 517.) Lopez Garcia reports that

> [w]hen the surfactant concentration was higher than about 0.20%, the slurries gave rise to abundant foam and small air bubbles were trapped in the sample loop of the FI manifold, making reproducibility worse. Although this problem could be overcome by adding several drops of antifoaming agent ['Antifoam A' (Fluka)] to the slurry, it was also noted that Triton X-100 decreased the absorption signal of copper, both for aqueous solutions and for slurries, and hence this surfactant was not employed.

(Lopez Garcia 518.) Thus, the problem addressed by adding anti-foam reagents in Lopez Garcia were bubbles in the FI manifold. This problem is not reasonably pertinent to the problem of optical detection as described in Appellant's Specification because introducing samples through an FI manifold to a flame is not related to optical detection of a fluorescent signal in liquid reaction mixture.

Although we are not persuaded by Appellant's arguments that Lopez Garcia is non-analogous art because it does not teach measurements of polymerase, or any other enzyme, activity, we are persuaded that it is non-analogous because it is not pertinent to the problems of bubble interference in the optical detection of a fluorescent signal in RT-PCR. Accordingly, we are persuaded that Lopez Garcia does not contribute to the obviousness of the method recited in Appellant's claim 43.

In affirming an obviousness rejection, the Board may rely upon fewer than all the references cited by the Examiner. *See In re May*, 574 F.2d 1082, 1090 (CCPA 1978); *In re Kronig*, 539 F.2d 1300, 1304 (CCPA 1976). Despite the inappropriateness of the citation to Lopez Garcia, we are not

persuaded that the Examiner erred in rejecting claim 43 because McMillan provides a sufficient teaching of the problem addressed by the recited method – interference of bubbles caused by detergents in optical measurements for PCR data analysis. The Examiner finds that one of ordinary skill in the art would have readily understood that bubbles interfere with optical detection in RT-PCR from the teachings of McMillan and thus, "[t]he motivation to use an antifoam is provided by the combined teachings of McMillan et al. and Lopez Garcia et al." (Ans. 13.) Thus, we are persuaded that even if Lopez Garcia does not provide a reason why one of ordinary skill in the art would have modified, the method taught in Li, McMillan does.

Appellant argues that ordinarily skilled artisans would not have been motivated to add additional components to RT-PCR reactions because at the time of filing, the general state of the art was to simplify reactions by reducing the number of components in RT-PCR based on the asserted known sensitivity of these reactions. (*See* Appeal Br. 15–16.) Appellant cites to Higuchi[12] in support of this argument. Higuchi was cited by the Examiner in a prior Office Action to show that it was known that bubbles interfere with optical detection in real-time RT-PCR. (*See* Office Action of January 3, 2017, 8, citing Higuchi 6 ("Small air bubbles trapped under the oil overlay will expand and contract during thermal cycling and can cause signal variations."), 8, 16–17.) Appellant argues that Higuchi's teaching to centrifuge the reaction mixture to remove problematic air bubbles, instead of

[12] Higuchi and Watson, *Kinetic PCR Analysis Using a CCD-camera and without Using Oligonucleotide Probes*, PCR Methods Manual, 1–24 (1999).

adding another component to the reaction mixture, demonstrates the motivation in the art to simplify reaction mixtures. (*See* Appeal Br. 15–16.)

We are not persuaded by this argument because Appellant fails to direct us to evidence that actually cautions against adding additional components to a PCR reaction mixture to remove bubbles or for any other reason. That Higuchi, and McMillan, teach other remedies for the problems caused by air bubbles does not indicate that the claimed remedy would not have also been obvious.

Appellant argues further there would not have been a reasonable expectation of success in adding anti-foam reagent to RT-PCR assays. (*See* Appeal Br. 26–33.) Appellant argues, first, that Lopez Garcia does not provide an expectation of success because the anti-foam reagent worsened the reaction being tested and was ultimately not chosen as the solution to the problem caused by bubbles. (*See id.* at 27–28.) As discussed above, we are not persuaded that Lopez Garcia contributes to the prima facie case for obviousness because the problem caused by bubbles in that reference is not pertinent to the problem of Appellant's claimed method. We are similarly not persuaded that even if Lopez Garcia ultimately relied on a different *way* to reduce bubbles, those of ordinary skill in the art would not have reasonably expected anti-foam to solve problems caused by bubbles in RT-PCR by similarly reducing bubbles because the methods and problems of Lopez Garcia are not analogous to the claimed methods.

Appellant argues further that there would not have been a reasonable expectation of success in adding anti-foam to one system when it had been added to what Appellant characterizes as a completely different system. (*See* Appeal Br. 28.) Appellant argues that the thermal strand displacement

amplification ("SDA") assay of Durmowicz uses different conditions and amplification processes than PCR. (*See id.*, citing Durmowicz, cols. 10–11 (comparing strand displacement amplification and PCR.) According to Appellant, because Durmowicz uses the polymerase Bst in SDA assays, which is known to be thermally unstable under PCR conditions, and conducts these assays without a detergent, one of ordinary skill in the art would have known that the components of SDA and PCR assays could not be interchanged with a reasonable expectation of success. (*See* Appeal Br. 28–29.) Appellant argues that one of ordinary skill in the art would have not have had a reasonable expectation of success using the conditions of thermolabile polymerase with a thermostable polymerase. (*See id.*)

We are not persuaded by this argument because "[o]nly a reasonable expectation of success, not absolute predictability, is necessary for a conclusion of obviousness." *In re Longi*, 759 F.2d 887, 897 (Fed. Cir. 1985). Appellant focuses on the differences between the Bst polymerase of Durmowicz and the thermostable polymerases used in PCR, but both enzymes are DNA polymerases and carry out similar functions in presumably similar ways.

Appellant argues that components used with SDA were known to be incompatible with PCR, specifically the acetylated BSA included in Example 14 of Durmowicz, which had been shown to be inhibitory to PCR. (*See* Appeal Br. 29–30, citing Kreader.[13]) The rejection is not based on

[13] Kreader, "Relief of Amplification Inhibition in PCR with Bovine Serum Albumin or T4 Gene 32 Protein," Applied Environmental Microbiology, 1102–06 (1996).

using all of the components of SDA as taught in Durmowicz, but rather on the suggestion that anti-foam reagents could be used successfully with the polymerase in RT-PCR.

> The test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art.

In re Keller, 642 F.2d 413, 425 (CCPA 1981).

The facts before us are similar to those in *In re Droge*, 695 F.3d 1334, 1337–38 (Fed. Cir. 2012), where a reasonable expectation of success of a method mediating sequence specific recombination of DNA in eukaryotic cells with a modified enzyme was found because the normal enzyme had previously been used to mediate similar recombination. Although one of ordinary skill in the art would not have known that the modified enzyme would work in the same way as the normal enzyme, the fact that both enzymes shared structural and functional similarities, indicated a reasonable expectation of success. Similarly, because the Bst polymerase of Durmowicz and the thermostable polymerases of the claimed RT-PCR methods ultimately achieve the same goal in generally the same way – polymerization of DNA templates by covalent linkage of nucleic acids – there would have been a sufficient basis for one of ordinary skill to have expected success.

> When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue

> the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that it was obvious under § 103.

KSR, 550 U.S. at 421.

Appellant also argues that Example V of the Specification is evidence that substances that inhibit enzyme activity can limit the use of RT-PCR and that it is not as simple as choosing any anti-foam reagent at any concentration to improve a PCR reaction itself. (*See* Appeal Br. 52–53; *see* Reply Br. 7.) Example V states: "Anti-foam concentration of 0.1% was inhibitory to PCR amplification with the selected set of anti-foam compounds resulting in either no amplification or delayed threshold cycle for product detection. Anti-foam concentrations of 0.01% or lower did not inhibit PCR amplification." (Spec. 28:22–24.)

Although we agree with Appellant that it would not be absolutely predictable that anti-foam reagent could be successfully added to RT-PCR, because it was successful with at least one DNA polymerase, one of ordinary skill in the art would have had a reasonable expectation of success to at least try anti-foam reagent in PCR. Furthermore, as the Examiner indicates, routine optimization is not considered inventive and Example V demonstrates that selecting the antifoams and determining their optimal concentrations require nothing more than routine experimentation. (Ans. 27.) Appellant's Specification states: "One skilled in the art will recognize that selection of an anti-foam reagent and choice of appropriate concentration for in vitro enzymatic reactions as described in the present

invention may require some routine experimentation." (Spec. 18:27–29 (emphasis omitted).)

Appellant argues that because McMillan does not teach or suggest adding anti-foam reagent, even though it recognizes the problems bubbles can present, those of ordinary skill in the art would have understood that RT-PCR is a complex reaction that is unpredictable and would not have known whether thermostable polymerases and anti-foam agents were compatible. (*See* Appeal Br. 31.) We are not persuaded by this argument because McMillan's alternative solution to the problem of bubbles (using a diamond-shaped chamber, *see* McMillan 19:34–37) says nothing about whether an anti-foam solution would also have been expected to succeed. McMillan need not have presented every solution to the problem it discusses for any one solution to have been reasonably expected to succeed.

Appellant argues that Lopez Garcia provides evidence that the combination of an anti-foam reagent and detergent is unpredictable and unsuitable at least in slurry flame atomic absorption spectrometry reactions. (*See* Appeal Br. 14, 32.) We are not persuaded that the ultimate determination in Lopez Garcia to remove the foaming detergent from sample is a teaching that anti-foam would not have been reasonably expected to address the problems created by bubbles in RT-PCR. Lopez Garcia states:

> Although this problem [of trapped bubbles] could be overcome by adding several drops of antifoaming agent ['Antifoam A' (Fluka)] to the slurry, it was also noted that Triton X-100 decreased the absorption signal of copper, both for aqueous solutions and for slurries and hence this surfactant was not employed.

(Lopez Garcia 518.) Thus, Lopez Garcia expressly teaches that a problem caused by bubbles could be solved with anti-foam reagent. One of ordinary skill in the art would have known that removing detergent is not an option for RT-PCR. (*See* Spec. 3:34–4:1 ("The bubbles likely are caused by the presence of non-ionic polymeric detergents, which are a necessary component of the amplification reaction.").) Thus, to the extent that Lopez Garcia is relevant to the issues of obviousness of the method of claim 43, we are not persuaded that it is evidence that using anti-foam in RT-PCR would have been unpredictable. Instead, Lopez Garcia teaches that anti-foam is a reliable way to reduce bubbles.

Appellant argues that the rejection is based on impermissible hindsight because

> [n]owhere in any of the cited references is there any teaching that combines a thermostable polymerase enzyme with a detergent and an antifoam reagent in RTQPCR for optical detection wherein the combination of reagents and a method that would in fact lead to not only an improvement in the accuracy of optical detection of nucleic acids, but also result in an improvement in the kinetics of transcription by the thermostable polymerase.

(Appeal Br. 45.) As discussed above, we are persuaded that one of ordinary skill in the art would have modified the RT-PCR assay of Li by adding anti-foam reagent to reduce the negative effects of bubbles on optical detection taught in McMillan. We are not persuaded that the method of claim 43 is not obvious because the Examiner has not cited a single reference that teaches including anti-foam reagent with a thermostable polymerase in RT-PCR. We are also not persuaded that any effects of adding anti-foam reagent, such as any improvement in the kinetics of transcription, would

have been unexpected and therefore would indicate that the claimed method is not obvious.

In summary, Appellant's arguments do not persuade us that the method claim 43 produces results that would have been unexpected by one of ordinary skill in the art at the time of filing. Nor do Appellant's arguments persuade us that adding anti-foam reagent to RT-PCR when it was known that bubbles present a problem would not have been obvious over the teachings of the prior art or that there are any errors in the Examiner's prima facie case for obviousness. Accordingly, we are not persuaded that the Examiner erred in rejecting claim 43.

Appellant does not raise separate arguments against the rejection of independent claim 52 or of the claims that depend on claim 43 or 52. Accordingly, we are not persuaded that the Examiner erred in rejecting these claims.

Examiner's rejection of claims 49 and 53 under 35 U.S.C. § 103 over Li, McMillan, Lopez Garcia, Durmowicz, Kyle, and Wierenga

After withdrawing the rejection of claims 43–47, 49, 52, 53, 56–61, 62, and 64 as being obvious over Li, McMillan, Lopez Garcia, Durmowicz, and Kyle (*see* Ans. 6–7), the Examiner entered a new ground of rejection under 35 U.S.C. § 103 over Li, McMillan, Lopez Garcia, Durmowicz, Kyle, and Wierenga that applies to claims 49 and 53. (*See id.* at 7–8.)

Claim 49 recites: "The method according to claim 43, wherein said polymerase chain reaction is carried out in the presence of an effective amount of at least two anti-foam reagents." (Appeal Br. 78.) Claim 53 depends on claim 52 and recites a composition comprising at least two anti-foam reagents. (*See id.*)

As the Examiner finds, Wierenga teaches that silicone-based anti-foam reagents are not that effective and that the addition of organic anti-foam reagents results in a synergistic anti-foaming combination. (*See* Wierenga 1:38–51, 1:66–2:3; *see* Ans. 8.) Wierenga teaches that carboxylated poly(oxyalkylated) alcohol cosurfactants have anti-foaming properties and act synergistically to inhibit foam formation. (*See* Wierenga 4:1–16; *see* Ans. 34.) The Examiner finds that it would have been obvious to combine the anti-foam reagent 1520-US with a second organic anti-foam reagent because of the synergistic combination taught in Wierenga. (*See* Ans. 8.)

Appellant first argues that the Examiner erred by first pointing to the failure of the Sigma Catalog to teach organic anti-foam reagents. (*See* Appeal Br. 56–58.) Because the Examiner's new ground of rejection does not rely on the Sigma Catalog, we are not persuaded by these arguments and do not address them.

Appellant argues that Wierenga does not teach a combination of anti-foams, but teaches the combination of silicone anti-foaming agent and a carboxylated linear alkoxylated alcohol, referred to as CS-I, which Appellant argues is not an anti-foam reagent. (*See* Appeal Br. 58.) Appellant does not address the teaching in Wierenga that carboxylated poly(oxyalkylated) alcohol cosurfactants have anti-foaming properties and act synergistically with silicone anti-foaming reagents. (*See* Wierenga 4:1–16.) Accordingly, we are unpersuaded by Appellant's argument.

Appellant raises other arguments against the rejection of claims 49 and 53 that are similar to the arguments addressed above made in regard to

the rejection of claim 43. (*See* Appeal Br. 62–65.) For the reasons discussed above, we are not persuaded by these arguments.

The Examiner's rejection of claims 43–48, 52, and 56–66 over Li, McMillan, Lopez Garcia, Durmowicz, Kyle, and Blaschke

Appellant's claim 48 recites: "The method according to claim 43, comprising detecting said product using a fluorescent nucleic acid-binding dye." (Appeal Br. 78.) Claim 62 similarly recites the composition of claim 59 comprising detection by fluorescent nucleic acid binding dye. (*See id.* at 80.)

The Examiner finds that Blaschke teaches real-time RT-PCR performed with either TaqMan probes or nucleic acid-binding dyes. (*See* Ans. 6, citing Blaschke 80 ("Recently, real-time PCR has become available, using either labelled sequence-specific probes (e.g., TaqMan®, Heid et al., 1996) or a fluorescent dye (e.g., SYBR Green, ethidium bromide, Higuchi et al. 1992) to monitor the formation of PCR products.").) Appellant does not contest this finding.

Appellant argues that Blaschke fails to cure the alleged deficiencies of the other cited prior art. (*See* Appeal Br. 66–74.) Because we do not agree that the prior art cited by the Examiner is deficient, we are not persuaded by Appellant's arguments, as discussed above.

Conclusion

Upon consideration of the record and for the reasons given, we affirm the Examiner's rejection.

In summary:

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
43–47, 52, 56–61, 63, 64	103	Li, McMillan, Lopez Garcia, Durmowicz, Kyle	43–47, 52, 56–61, 63, 64	
43–48, 52, 56–64	103	Li, McMillan, Lopez Garcia, Durmowicz, Kyle, Blaschke	43–48, 52, 56–64	
49, 53	103	Li, McMillan, Lopez Garcia, Durmowicz, Kyle, Wierenga	49, 53	
Overall Outcome			43–49, 52, 53, 56–64	

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136.

AFFIRMED