UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Laboratory Corporation of America Holdings
Petitioner

v.

Ravgen, Inc.
Patent Owner

Case IPR2021-00902

U.S. Patent No. 7,332,277

Standard Acknowledgment for Access to Protective Order Material

I_____, affirm that I have read the Protective Order; that I will abide by its terms; that I will use the confidential information only in connection with this proceeding and for no other purpose; that I will only allow access to support staff who are reasonably necessary to assist me in this proceeding; that prior to any

disclosure to such support staff I informed or will inform them of the requirements of the Protective Order; that I am personally responsible for the requirements of the terms of the Protective Order and I agree to submit to the jurisdiction of the Office and the United States District Court for the Eastern District of Virginia for purposes of enforcing the terms of the Protective Order and providing remedies for its breach.

_____

[Signature]

Printed Name:_____

Date:_____

STIPULATED PROTECTIVE ORDER

The following Stipulated Protective Order will govern the filing and treatment of confidential information in the proceeding:

Stipulated Protective Order

This protective order governs the treatment and filing of confidential information, including documents and testimony.

1. Confidential information shall be clearly marked "PROTECTIVE ORDER MATERIAL."

2. Access to confidential information is limited to the following individuals who have executed the acknowledgment appended to this order:

(A) Parties. Persons who are owners of a patent involved in the proceeding and other persons who are named parties to the proceeding.

(B) Party Representatives. Representatives of record for a party in the proceeding.

(C) Experts. Retained experts of a party in the proceeding who further certify in the Acknowledgement that they are not a competitor to any party, or a consultant for, or employed by, such a competitor with respect to the subject matter of the proceeding.

(D) In-house counsel. Up to a maximum of two (2) in-house counsel of a party.

(E) Support Personnel. Administrative assistants, clerical staff, court reporters and other support personnel of the foregoing persons who are reasonably necessary to assist those persons in the proceeding shall not be required to sign an Acknowledgement, but shall be informed of the terms and requirements of the Protective Order by the person they are supporting who receives confidential information.

(F) The Office. Employees and representatives of the United States Patent and Trademark Office who have a need for access to the confidential information shall have such access without the requirement to sign an Acknowledgement. Such employees and representatives shall include the Director, members of the Board and their clerical staff, other support personnel, court reporters, and other persons acting on behalf of the Office.

3. Employees (e.g., corporate officers), consultants, or other persons performing work for a party, other than those persons identified above in (2)(A)–(E), shall be extended access to confidential information only upon agreement of the parties or by order of the Board upon a motion brought by the party seeking to disclose confidential information to that person and after signing the Acknowledgment. The party opposing disclosure to that person shall have the burden of proving that such person should be restricted from access to confidential information.

4. Persons receiving confidential information shall use reasonable efforts to maintain the confidentiality of the information, including:

(A) Maintaining such information in a secure location to which persons not authorized to receive the information shall not have access;

(B) Otherwise using reasonable efforts to maintain the confidentiality of the information, which efforts shall be no less rigorous than those the recipient uses to maintain the confidentiality of information not received from the disclosing party;

(C) Ensuring that support personnel of the recipient who have access to the confidential information understand and abide by the obligation to maintain the confidentiality of information received that is designated as confidential; and

(D) Limiting the copying of confidential information to a reasonable number of copies needed for conduct of the proceeding and maintaining a record of the locations of such copies.

5. Persons receiving confidential information shall use the following procedures to maintain the confidentiality of the information:

(A) Documents and Information Filed With the Board.

(i) A party may file documents or information with the Board along with a Motion to Seal. The Motion to Seal should provide a non-confidential

description of the nature of the confidential information that is under seal, and set forth the reasons why the information is confidential and should not be made available to the public. A party may challenge the confidentiality of the information by opposing the Motion to Seal. The documents or information shall remain under seal unless the Board determines that some or all of it does not qualify for confidential treatment.

(ii) Where confidentiality is alleged as to some but not all of the information submitted to the Board, the submitting party shall file confidential and non-confidential versions of its submission, together with a Motion to Seal the confidential version setting forth the reasons why the information redacted from the non-confidential version is confidential and should not be made available to the public. A party may challenge the confidentiality of the information by opposing the Motion to Seal. The non-confidential version of the submission shall clearly indicate the locations of information that has been redacted. The confidential version of the submission shall be filed under seal. The redacted information shall remain under seal unless the Board determines that some or all of the redacted information does not qualify for confidential treatment.

(B) Documents and Information Exchanged Among the Parties. Documents (including deposition transcripts) and other information

designated as confidential that are disclosed to another party during discovery or other proceedings before the Board shall be clearly marked as "PROTECTIVE ORDER MATERIAL" and shall be produced in a manner that maintains its confidentiality.

6. Within 60 days after the final disposition of this action, including the exhaustion of all appeals and motions, each party receiving confidential information must return, or certify the destruction of, all copies of the confidential information to the producing party.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Laboratory Corporation of America Holdings
Petitioner

v.

Ravgen, Inc.
Patent Owner

Case IPR2021-01054

U.S. Patent No. 7,332,277

Standard Acknowledgment for Access to Protective Order Material

I_____, affirm that I have read the Protective Order; that I will abide by its terms; that I will use the confidential information only in connection with this proceeding and for no other purpose; that I will only allow access to support staff who are reasonably necessary to assist me in this proceeding; that prior to any

disclosure to such support staff I informed or will inform them of the requirements of the Protective Order; that I am personally responsible for the requirements of the terms of the Protective Order and I agree to submit to the jurisdiction of the Office and the United States District Court for the Eastern District of Virginia for purposes of enforcing the terms of the Protective Order and providing remedies for its breach.

_____

[Signature]

Printed Name:_____

Date:_____

Trials@uspto.gov
571-272-7822

Paper 55
Entered: November 1, 2022

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

LABORATORY CORPORATION OF AMERICA HOLDINGS,
Petitioner,

v.

RAVGEN, INC.,
Patent Owner.

IPR2021-00902
Patent 7,332,277 B2

Before ZHENYU YANG, TIMOTHY G. MAJORS, and DAVID COTTA, *Administrative Patent Judges*.

MAJORS, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining No Challenged Claims Unpatentable
35 U.S.C. § 318(a)
Granting Patent Owner's Motion for Entry of Protective Order and to Seal;
Granting Petitioner's Motion to Seal
37 C.F.R. §§ 42.14, 42.54
Dismissing Petitioner's Motion to Exclude
37 C.F.R. § 42.64(c)

I. INTRODUCTION

Laboratory Corporation of America Holdings ("Petitioner" or "Labcorp"),[1] on May 3, 2021, filed a Petition to institute *inter partes* review of claims 81–96 and 133 of U.S. Patent No. 7,332,277 B2 (Ex. 1001, "the '277 patent"). Paper 1 ("Pet." or "Petition"). We instituted trial on November 5, 2021. Paper 13 ("Inst. Dec."). During trial, Ravgen, Inc. ("Patent Owner")[2] filed a Patent Owner Response. Paper 21 ("PO Resp."). Later filings include Petitioner's Reply (Paper 30 ("Pet. Reply")) and Patent Owner's Sur-Reply (Paper 40 ("PO Sur-Reply")). An oral hearing was held on July 28, 2022, and a transcript is entered in the record. Paper 54 ("Tr.").

In addition, we authorized the parties to submit post-hearing briefs regarding the interpretation of a handful of dependent claims (Paper 51, Paper 52, Ex. 3004). Patent Owner also filed a motion for entry of a protective order and to seal (Paper 22), and Petitioner filed a motion to seal (Paper 31); both motions were unopposed. Petitioner filed a motion to exclude Exhibit 2301 (*see* Paper 45 ("Mot. to Exclude") and Paper 48 ("Mot. Reply")), which Patent Owner opposed (Paper 47 ("Opp.")).

We have jurisdiction under 35 U.S.C. § 6(b). After considering the parties' arguments and evidence, we determine that Petitioner has not proved by a preponderance of the evidence that the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e). Our reasoning is explained below, and we issue this Final Written Decision under 35 U.S.C. § 318(a).

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[1] Petitioner identifies itself as the real party-in-interest. Pet. 1.
[2] Patent Owner identifies itself as the real party-in-interest. Paper 6, 1.

A. Related Patents & Proceedings

The '277 patent issued February 19, 2008, from U.S. Patent Application No. 10/661,165 ("the '165 Application"), filed September 11, 2003. Ex. 1001, codes (21), (22), (45). The '165 Application is a continuation-in-part ("CIP") of Application No. PCT/US03/06198 filed February 28, 2003 ("the '198 PCT" (Ex. 1007)), which claims priority to other applications including U.S. Provisional Application No. 60/378,354, filed May 8, 2002 ("the '354 Provisional" (Ex. 1011)). *Id.* at codes (60), (63); *see also id.* at 1:7–25. The '165 Application is also a CIP of Application No. PCT/US03/27308, filed August 29, 2003 ("the '308 PCT" (Ex. 1009)). *Id.* at 1:14–16. Related U.S. Patent No. 7,727,720 ("the '720 patent") issued on June 1, 2010, and claims priority to some of the same ancestral applications as the '277 patent. Ex. 2041, 1, 4.[3]

The parties identify multiple lawsuits involving the '277 patent. Pet. 1; Paper 6, 1; Paper 16, 1. Those lawsuits include: *Ravgen, Inc. v. Laboratory Corp. of America Holdings*, No. 6:20-cv-00969-ADA (W.D. Tex.); *Ravgen, Inc. v. Quest Diagnostics Inc.*, No. 2:21-cv-09011-RGK-GJS (C.D. Cal.); and *Ravgen, Inc. v. Natera, Inc. and NSTX, Inc.*, No. 1:20-cv-00692-ADA (W.D. Tex.). Paper 6, 1 (listing other lawsuits filed by Patent Owner against, e.g., PerkinElmer, Inc., and Myriad Genetics, Inc.).

[3] For some exhibits herein, we cite the page numbers added to the exhibit copy; we may also use other citation formats (e.g., column and line, paragraph numbers, or original pagination) for some exhibits.

Petitioner and Patent Owner also identify other matters involving the '277 patent pending before the Patent Office. Pet. 2; Paper 6, 1; Paper 8, 1; Paper 12, 1. Different claims of the '277 patent than challenged here are challenged in IPR2021-01054 (filed by Labcorp). Claims of the '277 patent have also been challenged in IPR2021-00788, -00789 and -00790 (all filed by Quest),[4] IPR2021-01272 (filed by Illumina, Inc.) and IPR2021-01577 (filed by Streck, Inc.). Pet. 2; Paper 6, 1; Paper 8, 1; Paper 12, 1. We terminated IPR2021-00788 due to settlement prior to reaching a final written decision (IPR2021-00788, Paper 71), a final written decision in IPR2021-01054 is entered concurrent with this decision, and IPR2021-01272 and IPR2021-01577 are ongoing. In addition, Patent Owner identifies *Ex Parte* Reexamination Control No. 90/014,792 as related to the '277 patent. Paper 8, 1. That reexamination is stayed. Paper 24.

The related '720 patent has also been challenged in several matters before the Office: IPR2021-00791 (terminated); IPR2021-01026 (pending); IPR2021-01271 (pending); and *Ex Parte* Reexamination Control Nos. 90/014,703, and 90/014,869 (both stayed).

B. Asserted Grounds of Unpatentability

Petitioner asserts three grounds of unpatentability in this Petition (Pet. 8), which are provided in the table below:

Claims Challenged	35 U.S.C. §	Reference(s)/Basis
81–91, 94–96, 133	103(a)[5]	Chiu,[6] Bianchi[7]

[4] On October 19, 2021, we instituted trial in IPR2021-00788, Paper 23

Claims Challenged	35 U.S.C. §	Reference(s)/Basis
92, 93	103(a)	Chiu, Bianchi, Granger[8]
81–96, 133	103(a)	Chiu, Rao[9]

Petitioner relies on the declarations of Jeremy S. Edwards, Ph.D., among other evidence. Ex. 1002; Ex. 1047. Prior to institution, Patent Owner submitted a declaration from Dr. Glenn D. Prestwich, Ph.D. Ex. 2001. During trial, Patent Owner submitted and relies on a declaration from Dr. Brian Van Ness, Ph.D. (Ex. 2078), among other evidence.[10] The deposition testimony of Drs. Edwards (Exs. 2101, 2299) and Van Ness (Ex. 1045) is also of record.

(covering claims 55–63, 66–69, 80–94, 96, and 126–133), and denied institution in IPR2021-00789 (Paper 21) and IPR2021-00790 (Paper 21).

[5] The Leahy-Smith America Invents Act, Pub. L. No. 112-29, 125 Stat. 284 (2011) ("AIA"), amended 35 U.S.C. § 103. Based on the filing date of the '277 patent, we apply the pre-AIA version of § 103.

[6] Rossa W. K. Chiu et al., *Effects of Blood-Processing Protocols on Fetal and Total DNA Quantification in Maternal Plasma*, 47:9 CLINICAL CHEMISTRY 1607–1613 (2001) (Ex. 1003, "Chiu").

[7] Bianchi et al., U.S. 5,648,220, issued July 15, 1997 (Ex. 1004, "Bianchi").

[8] Granger et al., WO 95/01796, published January 19, 1995 (Ex. 1006, "Granger").

[9] Rao et al., WO 03/018757 A2, published March 6, 2003 (Ex. 1005, "Rao").

[10] The parties submit, for example, additional testimony related to products that are alleged to practice the claimed subject matter and asserted commercial success of such products. *See, e.g.*, Exs. 2080 (Declaration of Paul Meyer) and 2082 (Declaration of Jeffrey Chalmers, Ph.D.).

C. Technology Overview and the '277 Patent

The '277 patent relates to non-invasive methods for sampling DNA and detection of genetic disorders in a fetus. Ex. 1001, 1:31–39. The '277 patent explains that a variety of invasive and non-invasive techniques are available for prenatal diagnosis, including amniocentesis, fetal blood cells in maternal blood, and maternal serum alpha-feto protein. *Id.* at 2:53–57. According to the patent, however, "techniques that are non-invasive are less specific, and the techniques with high specificity and high sensitivity are highly invasive." *Id.* at 2:57–60; *see also id.* at 3:33–37 (citing an increased fetal mortality risk of about 0.5% with amniocentesis).

We provide a brief overview of the components of blood, which is helpful in understanding the challenged claims and the cited prior art. Blood is composed of plasma and other blood components that are suspended in plasma. Ex. 2001 ¶ 35; Ex. 2078 ¶ 22. The major blood components in plasma include red blood cells ("RBCs"), white blood cells ("WBCs") and platelets. *Id.* Although most DNA is typically found inside cells (within the cell membrane and nucleus), some DNA may also be found outside the cells circulating freely in the plasma. *Id.* ¶¶ 22–23. Such circulating DNA is known as "cell-free DNA" ("cfDNA"). *Id.* WBCs, unlike RBCs, include an individual's cellular DNA, and when WBCs are subjected to various stresses (e.g., biological, physical, or chemical), the WBCs may lyse and release additional DNA into the plasma. Ex. 2001 ¶¶ 37–38; *see also* Ex. 1002 ¶ 24 (discussing liberation of DNA from lysis of maternal cells).

By the late 1990s, and prior to the '277 patent, researchers had discovered that pregnant women have cell-free *fetal* DNA ("cffDNA") along

with maternal cfDNA circulating in maternal plasma. Ex. 2001 ¶¶ 36–37; Ex. 2078 ¶ 23. For example, in a study headed by Dr. Dennis Lo, researchers determined that cffDNA was present in maternal plasma in a range of about 3.4%–6.2% (as a percent of total circulating DNA), with the percentages corresponding to early and late pregnancy, respectively. Ex. 1002 ¶ 24 (citing Ex. 1021, 768). At about the same time, researchers determined that, although intact fetal *cells* may also be found in maternal plasma, most fetal DNA in maternal plasma exists in its cell-free form. *Id.*

To analyze cell-free DNA from blood, a blood sample is ordinarily collected (e.g., from a subject's vein), and then further processed. Ex. 2001 ¶ 38. A common mode of preparation for such blood samples involves the use of centrifugation to separate the cellular components and plasma within the sample. Ex. 1002 ¶ 23 (discussing methods to separate plasma from mononuclear cells, which cells are typically present in a "buffy layer" at the interface with the plasma layer following centrifugation); *see generally* Ex. 1003 (describing, *inter alia*, centrifugation and filtration techniques to separate the plasma and cellular fractions).

According to Petitioner's declarant, Dr. Edwards, when working with blood samples, it was known to add blood stabilizing compounds, especially to reduce effects of delayed processing. Ex. 1002 ¶¶ 21–22. Dr. Edwards testifies that known processing "techniques included the use of agents that stabilized the cells and/or analyte(s) and/or prevented coagulation [of the blood] so that samples could be tested, hours, days, or weeks after collection." *Id.* "For example, blood samples were commonly collected in

treated tubes, e.g., EDTA tubes or acid citrate dextrose (ACD) tubes." *Id.* (citing, e.g., Exs. 1003 and 1014).

The '277 patent acknowledges the prior non-invasive use of fetal cells and cell-free fetal DNA, both isolated from maternal blood, for prenatal diagnosis. Ex. 1001, 5:7–59. With regard to fetal cells, the patent notes that the "presence of fetal nucleated cells in maternal blood makes it possible to use these cells for noninvasive prenatal diagnosis," and that such "cells can be sorted and analyzed by a variety of techniques to look for particular DNA sequences." *Id.* at 5:8–13. Yet the patent states that "it is still difficult" to get many fetal cells from maternal blood and "[t]here may not be enough to reliably determine anomalies of the fetal karyotype or assay for other abnormalities." *Id.* at 5:30–34. The patent states that fetal DNA "has been detected and quantitated in maternal plasma and serum" and that "fetal DNA present in the maternal serum and plasma is comparable to the concentration of DNA obtained from fetal cell isolation protocols." *Id.* at 5:39–49. "However," according to the patent, "the diagnostic and clinical applications of circulating fetal DNA is limited to genes that are present in the fetus but not in the mother" and "a need still exists for a non-invasive method that can determine the sequence of fetal DNA and provide definitive diagnosis of chromosomal abnormalities in a fetus." *Id.* at 5:53–59.

The '277 patent describes a method that is said to increase the proportion or percentage of the cffDNA component in a sample from a pregnant female for subsequent analysis. According to the patent, the ability to detect chromosomal abnormalities has been "hindered by the low percentage of free fetal DNA" in maternal samples. Ex. 1001, 89:1–6.

"Increasing the percentage of free fetal DNA would enhance the detection" of trisomy and other genetic abnormalities. *Id.* at 89:6–11.

With the aim of increasing the percentage of cffDNA relative to circulating maternal DNA in a maternal sample, the '277 patent describes adding an agent that inhibits cell lysis. Ex. 1001, 219:38–44 (Example 15) ("[T]he use of cell lysis inhibitors, cell membrane stabilizers, or cross-linking reagents can be used to increase the percentage of fetal DNA in the maternal blood."); *id.* at 89:11–13 ("The percent of fetal DNA in plasma obtained from a pregnant female was determined both in the absence and presence of inhibitors of cell lysis"). The patent explains that, "[w]hile lysis of both maternal and fetal cells is inhibited, the vast majority of cells [in a maternal blood sample] are maternal, and thus by reducing the lysis of maternal cells, there is a relative increase in the percentage of free fetal DNA." *Id.* at 32:36–39. The patent identifies numerous agents as cell lysis inhibitors, cell membrane stabilizers, or cross-linking reagents. *See, e.g.*, *id.* at 31:57–32:21 (listing, for example, formaldehyde, formalin, cleavable crosslinkers, cholesterol, and glucose).

The '277 patent provides results on the use of formalin (i.e., formaldehyde in aqueous solution) as the lysis-inhibiting agent. Ex. 1001, 89:1–91:50 (Example 4). In Example 4, the patent describes collecting a 5 ml blood sample from a pregnant subject, separating the sample into two tubes (each containing EDTA[11]), and adding formaldehyde (25µl/ml) to one

[11] The '277 patent states that EDTA is a "magnesium chelator." Ex. 1001, 31:52–54.

of the tubes. *Id.* at 89:18–25. The samples were centrifuged and 800 µl of each maternal plasma sample was then used for DNA purification and further processing to determine the relative amount of cffDNA present. *Id.* at 89:25–91:13. According to the '277 patent, "the percentage of fetal DNA present in the sample that was treated with only EDTA was 1.56%" and the "percentage of fetal DNA present in the sample treated with formalin and EDTA was 25%." *Id.* at 91:14–20. The percent of total cffDNA in eighteen blood samples with and without formalin was then calculated, with the results (mean percentage cffDNA) provided in Table V. *Id.* at 91:35–43 (reporting, *inter alia*, 19.47% with formalin and 7.71% without formalin), 219:38–226:26 (Example 15).

D. Challenged Claims

Independent claim 81 and sixteen claims that depend (directly or indirectly) from claim 81 are challenged here. Claim 81 reads:

> 81. A method for preparing a sample for analysis comprising isolating free fetal nucleic acid from a the sample, wherein said sample comprises an agent that inhibits lysis of cells, if cells are present, and wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Ex. 1001, 474:52–57.

To illustrate some of the challenged dependent claims, claim 90 depends from claim 81 and adds, "said cell lysis inhibitor is selected from the group consisting of glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, formalin, and derivatives of formaldehyde." *Id.* at 475:15–

18. Claim 91 depends from claim 90, adding "said cell lysis inhibitor is formalin." *Id.* at 475:15–20.

E. Prosecution History

Challenged claim 81 corresponds to pending claim 87 in prosecution. Ex. 1025, 530.

The Examiner initially rejected pending claim 87 as anticipated by "the Lo Patent"[12] and also rejected claim 87 as obvious over the Lo Patent and certain other references. *Id.* at 1228–1232. Applicant argued in response that the Examiner had provided no evidence that EDTA in the Lo Patent's samples inhibits cell lysis, and, citing Example 4 of the application, argued that adding a lysis-inhibiting agent (i.e., formalin) solved a long-felt need and provided unexpected results. *Id.* at 1194–1196, 1199–1200.

The Examiner withdrew the rejections based on the Lo Patent, but entered new rejections for obviousness based on the combination of "Amicucci" or "Umansky," with "Kiessling" (citing Kiessling for its disclosure of formaldehyde as a cell fixative). *Id.* at 927–931, 958–961.

In Remarks dated May 30, 2007, applicant argued that there was no motivation to combine the newly cited references. *See, e.g.*, *id.* at 574. Applicant argued that the DNA analyzed in the methods of Umansky and Kiessling was "quite distinct" because Umansky analyzed cell-free fetal DNA circulating outside a cell, "while the DNA analyzed in Kiessling is in and/or is released from a fixed cell." *Id.*; *see also id.* at 593 (advancing

[12] Patent Publication No. WO 98/39474 (Ex. 2038 or the "Lo Patent").

similar argument for the Amicucci combination). Applicant also argued that the claimed method addressed a long-felt need and produced unexpected results. *Id.* at 573–574, 591–592. Specifically, applicant argued that its method was an alternative to invasive prenatal testing, and that, by adding formalin as an agent that inhibits lysis to the maternal sample, the percentage of cffDNA was 25%, compared to 1.56% without formalin. *Id.* at 574 (asserting that, based on prior reports, "the mean percentage of free fetal DNA in a maternal sample was expected to be about 3%").

The Examiner, on September 26, 2007, entered a Notice of Allowability covering claim 87 and the several other claims that ultimately issued. Ex. 1025, 523–525. The Examiner's Reasons for Allowance stated that the various claims are "deemed to be allowable in light of the applicant's amendment filed 30 MAY 07 and the persuasive argument(s) therein." *Id.* at 525.

II. ANALYSIS

A. Principles of Law

"In an [*inter partes* review], the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable." *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify "with particularity . . . the evidence that supports the grounds for the challenge to each claim")).

A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the subject matter sought to be patented and the prior art are such

that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which that subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) secondary considerations of nonobviousness when presented. *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966). When evaluating a combination of teachings, we must also "determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *KSR*, 550 U.S. at 418 (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)). Whether a combination of elements produces a predictable result weighs in the ultimate determination of obviousness. *Id.* at 416–417.

B. Person of Ordinary Skill in the Art ("POSA")

In determining the level of skill in the art, we consider the problems encountered in the art, the art's solutions to those problems, the rapidity with which innovations are made, the sophistication of the technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus., Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986).

Petitioner contends:

> A person of ordinary skill in the art (POSITA) would have had an advanced degree (e.g., M.S. or Ph.D.) in Chemistry, Biochemistry, Molecular Biology, Genetics, Bioengineering, Chemical Engineering, or a related discipline, and at least 2-3 years of experience in a research or clinical laboratory. . . . In

> addition, a POSITA would have been familiar with the available techniques for optimizing biological samples to be used in various laboratory analyses, such as for detection of DNA, and would have been familiar with the relevant scientific field and its literature at the time when the application was filed, in particular, literature regarding the detection of cell-free nucleic acids.

Pet. 18 (citing Ex. 1002 ¶¶ 12–13). Patent Owner's proposal states that a POSA would have had "a M.D. and/or a Ph.D. in a related area such as genetics, biochemistry, molecular biology, cell biology, or microbiology and at least one to two years of work in one of those related areas." PO Resp. 7. Patent Owner proposes that a POSA could, alternatively, "have a Bachelor's degree in one of the foregoing areas and at least three to four years of work in" such areas. *Id.* (citing Ex. 2078 ¶¶ 16–21).

Patent Owner's proposed POSA level is too broad. Under that proposal, an individual with, for example, an undergraduate degree in microbiology and three years' work experience studying the habitats of bacteria might qualify as a POSA. It is not clear that such person would have sufficient, relevant experience in the detection of chromosomal abnormalities, especially through non-invasive methods for detecting fetal genetic abnormalities in maternal samples, as described in the '277 patent and the prior art here. *See, e.g.*, Ex. 1001, 1:31–5:59 (Field of Invention and Background Art); Ex. 1003 (Chiu). Petitioner's proposal of the POSA level is more precise because it requires familiarity with techniques for detecting cell-free DNA in biological samples, which is relevant to the prior art and the claimed technology. Petitioner's proposal also appears to be more consistent with the cited prior art. *Okajima v. Bourdeau*, 261 F.3d 1350,

1355 (Fed. Cir. 2001) (explaining that specific findings regarding ordinary skill level are not required where the prior art itself reflects an appropriate level and a need for testimony is not shown). Accordingly, we adopt Petitioner's POSA level here, but note that our other determinations on this record would not change under Patent Owner's POSA level.[13]

C. Claim Construction

We interpret a claim "using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. 282(b)." 37 C.F.R. § 42.100(b) (2020). Under this standard, we construe the claim "in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." *Id.*

Petitioner does not identify, in the Petition, any terms for which it is seeking express claim construction. Pet. 9. Patent Owner, in its Response, "reserve[d] the right to address claim construction," but similarly did not request interpretation of any claims. PO Resp. 7 n.2.

The parties later disputed whether certain dependent claims (i.e., claims 90, 91, and 133) *require* specific agents, such as formaldehyde or formaldehyde derivatives (Patent Owner's position), or more broadly also encompass any "membrane stabilizer" (Petitioner's position). Pet. Reply 5

[13] Patent Owner's declarant, Dr. Van Ness, testifies that his opinions would not change under either POSA level. Ex. 2078 ¶ 21.

n.3; PO Sur-Reply 14–15; *see generally* Papers 51 and 52.[14] It is not necessary for us to further construe those dependent claims to resolve this case. Petitioner relies on formaldehyde or formaldehyde donors, as disclosed in Bianchi, Granger, and Rao, to satisfy the claimed "agent" term, and urges the addition of such compounds to Chiu's method for isolating and detecting cffDNA in a maternal sample. There is no dispute on this record that formaldehyde and formaldehyde donors meet the claimed "agent" term. We are, thus, able to determine if the asserted art discloses an "agent" as claimed and whether a POSA would have been motivated to add formaldehyde or a formaldehyde donor to Chiu, which issue we find is decisive here, without further interpreting dependent claims 90, 91, or 133. *See Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co. Ltd.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms that are in controversy, and only to the extent necessary to resolve the controversy.") (internal quotation marks omitted).

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[14] The disagreement on interpretation of this handful of dependent claims arose in relation to Rao's prior-art status for those claims (Pet. Reply 5 n.3; PO Sur-Reply 14–15), and Patent Owner's nexus showing for secondary considerations of nonobviousness (*id.* at 21). *See* Pet. Reply 3 n.2 (noting the priority "arguments are only relevant to Ground 3" because the prior art status of Chiu, Bianchi, or Granger is uncontested). But, as we discuss below, Petitioner's challenge to the claims fails on this record regardless of the disagreement on Rao's prior art status and, because Petitioner has not made the necessary threshold showing that a POSA would have combined the asserted art in the manner proposed, we need not reach secondary considerations of nonobviousness.

D. Overview of the Asserted Prior Art

1. Chiu (Exhibit 1003)

Chiu is an article about molecular diagnostics and genetics, published in Clinical Chemistry in 2001. *See generally* Ex. 1003. Chiu relates to a study on the effects of blood-processing protocols on the quantification of cell-free fetal and total DNA in maternal plasma. *Id.* at 1607–1608 ("[I]t is the objective of this study to investigate the effects of different blood-processing protocols on the quantitative analysis of total and fetal DNA in maternal plasma, as well as the effect on the relative proportions of cellular and cell-free DNA.").

Chiu discloses that "the discovery of fetal DNA in maternal plasma and serum in 1997 . . . [and] numerous reports have confirmed its potential application for noninvasive prenatal diagnosis." *Id.* at 1607. Citing prior studies, Chiu reports that "it has been shown that fetal DNA represents a substantial portion of the total DNA in maternal plasma, contributing ~ 3.4% and ~ 6.2% of total plasma DNA in early and late pregnancy, respectively." *Id.* Based on such prior investigations, Chiu addresses "whether plasma is truly acellular" and "whether fetal DNA circulates predominately in a cellular or cell-free form in maternal plasma." *Id.* at 1608; *see also id.* at 1607 (disclosing, as background, that "[r]ecently, apoptotic cells have been found in plasma obtained by centrifugation of blood from pregnant women, raising the question of what constitutes plasma and whether plasma is truly cell free"). Because studies may rely on quantification of fetal DNA in maternal plasma, Chiu discloses that "it would be of prime importance to

investigate whether the apparent maternal plasma DNA concentration would be affected by different sample processing protocols." *Id.* at 1608.

Chiu discloses the use of different protocols to process blood samples and separate maternal plasma; those protocols include centrifugation, microcentrifugation, and filtration. *Id.* at 1608–09. Certain genes (β-globin and *SRY*)[15] in the separated plasma were then isolated and amplified via PCR for determination of the concentrations (genome-equivalents/mL) of those genes in the samples. *Id.*

From the data, Chiu discloses that "different blood-processing protocols have a significant impact on the quantification of *β-globin*, but not *SRY* sequences in plasma." *Id.* at 1612. "In other words, by altering the blood-processing protocol, quantification of total, but not fetal, DNA is affected." *Id.* Chiu explains, for example, that "centrifugation alone, by various speeds (1600*g* and 800*g*) led to total DNA concentrations that were significantly different and higher than those of filtered plasma ($P < 0.05$)." *Id.* Chiu teaches, "[t]herefore, it can be deduced that despite centrifugation, some of the maternal cells could remain in plasma, leading to an increase in the total DNA in plasma." *Id.* ("[C]entrifugation alone is not effective in removing all of the cells in plasma, and the number of cells that remain in plasma after processing is variable."). Chiu teaches that the "lack of difference in fetal DNA concentration among the different [sample-

[15] These genes could be used as proxies in Chiu's methods for determining the amount of fetal to total DNA because the β-globin gene is present in maternal and fetal DNA, and the *SRY* gene only in the fetal DNA. Ex. 1003, 1608, 1612.

processing] treatment groups . . . suggests that most of the fetal DNA circulates in an extracellular form." *Id.* ("[I]ntact fetal cells contribute only a very small proportion of the quantifiable fetal DNA"); *see also id.* (disclosing that "fetal cells are detectable at a frequency of . . . ~ 2 fetal cells/mL of Percoll-derived maternal plasma"). Chiu concludes that "[d]ifferent protocols of blood sample processing impart a significant effect on the quantification of total DNA in maternal plasma." *Id.* at 1613.

Although Chiu indicates that an initial centrifugation may not, alone, be sufficient to remove cells from the plasma samples, Chiu teaches that cell-free samples may be obtained with additional physical processing steps—filtration or microcentrifugation. *Id.* at 1609–13. More specifically, Chiu discloses that "[p]lasma filtration by a submicron filter is used to remove residual cells that remain in plasma after the initial centrifugation step" and that "the DNA concentration in filtered plasma reflects the proportion of 'extracellular' fetal and total DNA in the blood sample." *Id.* at 1612. Chiu teaches that "[b]ecause plasma subjected to microcentrifugation [(i.e., centrifugation at 16,000*g*)] . . . consistently leads to a total DNA concentration that is statistically similar to that of filtered plasma, we infer that microcentrifugation is just as effective at generating cell-free plasma as filtration." *Id.*; *see also id.* at 1613 ("Virtually cell-free plasma can be obtained by centrifugation of blood samples, followed by filtration or microcentrifugation.").

Chiu closes its discussion with the following disclosure:

> By highlighting the importance of centrifugation protocols for plasma processing, our data have obvious bearing on this type of analysis [(analysis of free circulating nucleic acids, including,

> e.g., fetal DNA, and plasma DNA used for cancer diagnosis)]. As research in the field of circulating nucleic acids is growing rapidly for findings to be easily comparable across studies, some form of standardization needs to be agreed on.

Id.

2. Bianchi (Exhibit 1004)

Bianchi is a U.S. patent that issued in 1997. Ex. 1004, code (45). Bianchi discloses methods for labeling intracytoplasmic target molecules, e.g., a protein or a nucleic acid, in order to determine whether cells having such a target molecule are present in a sample. *Id.* at Abstr., 2:14–39 (listing target molecules, such as fetal hemoglobin that is characteristic of fetal cells (e.g., fetal nucleated erythrocytes) in a maternal blood sample). Bianchi describes the applicability of its methods to prenatal diagnosis ("allow[ing] single-cell genetic and chromosomal analysis which can be used for, e.g., prenatal diagnosis"). *Id*. at 2:66-3:1.

Bianchi teaches that there are two key features of its method. First, that the intracytoplasmic target molecule can be labeled within the cell. *Id.* at 3:24–32 ("First, the plasma membrane is sufficiently permeable so that a reagent capable of detectably labeling the target molecule is able to traverse the plasma membrane into the cytoplasm."). Second, that "the plasma membrane is sufficiently intact so that substantially all of the intracytoplasmic target molecule and the DNA of the target cell remain in the cell." *Id.* at 3:32–35.

Bianchi discloses use of a reagent system and sample treatment process for its "permeabilization method" where the cell preparation sample

is incubated in 2–8% (w/v) paraformaldehyde for between 10 minutes to 4 hours at, preferably, about 37° C. *Id.* at 3:37–42. The cell preparation suspected of including the target cell is "then is incubated permeabilized" in a solution containing alcohol (for example, incubating the sample in methanol:acetone at about 4° C). *Id.* at 3:44–53 (following permeabilization, the cells are washed and contacted with a labelling reagent, such as an antibody, which can be used for detecting the target cell from the cell preparation).

3. Rao (Exhibit 1005)

Rao is an international patent application that published on March 6, 2003. Ex. 1005, code (43).[16] Rao relates to "[c]ompositions and methods for stabilizing rare cells in blood specimens," and compositions that "serv[e] as cell fixatives" to "minimize losses of target cells (for example, circulating tumor cells [(CTCs)]) and formation of debris and aggregates from target cells." *Id.* at Abstr. (teaching that the cells are stabilized so the rare cells can be better detected or enumerated).

Rao discloses that tumor cells undergoing apoptosis have altered membrane permeability that may lead to the escape of DNA and other cellular components. *Id.* at 2:5–10 ("Such tumor cell debris may still bear epitopes that are characteristic of intact cells, and can lead to spurious increases in circulating cancer cells."). Rao teaches that "[l]eukocytes . . .

[16] Rao includes a priority claim to two U.S. provisional applications (Application No. 60/314,151, filed August 23, 2001 (Ex. 1048), and Application No. 60/369,628, filed April 3, 2002).

are known to be labile and diminish on storage," thus "increas[ing] the amount of cellular debris, derived from normal blood cells or proteins were found to interfere with the isolation and detection of rare target cells such as CTC." *Id.* at 2:14–17.

Rao discloses that "[t]here is a large body of published or patented art regarding the stability and stabilization of normal blood cells over time and several proprietary commercial stabilizers are available for preserving white blood cells." *Id.* at 3:4–8 (listing stabilizers, including "Cyto-Chex™ from Streck Laboratories"). Rao discloses that, "[d]espite the shortcomings of paraformaldehyde or reagents containing paraformaldehyde, formaldehyde, glutaraldehyde and glyoxal, such reagents are frequently used for fixing and stabilizing tumor cells in blood or histology specimens." *Id.* at 3:16–18.

Rao discloses that the "ideal" stabilizer preserves the target cells while minimizing interfering cellular debris in a sample. *Id.* at 7:20–24. Rao identifies "the formaldehyde donor imidazolidinyl urea" as being "effective" at a preferred concentration of 0.1–10% by volume of the specimen. *Id.* at 8:2–5, 22:1–14 (claim 1 composition, including a stabilizing agent), claim 6 (said stabilizing agent "is a formaldehyde donor")).

4. Granger (Exhibit 1006)

Granger is an international patent application that published January 19, 1995. Ex. 1006, code (43).

Granger relates to the stabilization of cells in blood. *Id.* at Abstr. Granger teaches "a method of stabilising the cellular constituents of whole blood, in particular leucocyte preparations formed for example from lysed whole blood, by the addition thereto of an effective amount of a heavy metal

compound." *Id.* at 5:14–18. Granger teaches that leucocytes may also be "preferably treated with a second stabilising agent which can be, for example, an aldehyde, preferably formaldehyde, most preferably paraformaldehyde." *Id.* at 12:1–5.

E. Obviousness over Chiu and Bianchi

Petitioner asserts that claims 81–91, 94–96, and 133 are unpatentable as obvious over the combination of Chiu and Bianchi. Pet. 18–36; *see id.* at 18–24 (independent claim 81), 24–36 (claims depending from claim 81).

As we discuss in our analysis below, this ground turns on whether Petitioner has met its burden to establish, by a preponderance of the evidence, that the POSA would have found it obvious to combine and modify the asserted cited art. More specifically, it turns on whether it would have been obvious to modify the maternal blood processing and cffDNA detection method of Chiu to include paraformaldehyde as a fixative agent, as allegedly disclosed in Bianchi, in the manner proposed by Petitioner.

We gave an overview of the asserted prior art above. Below, we review Petitioner's contentions on claim 81 and Patent Owner's counterarguments. We will then turn to our analysis.

1. Petitioner's Contentions on Claim 81

a) "A method for preparing a sample for analysis comprising"

Petitioner contends that Chiu teaches a method for preparing a sample (maternal blood) for analysis as recited in claim 81's preamble.[17] Pet. 20–21. Petitioner cites Chiu's teachings related to the processing of a maternal blood sample to produce plasma, such as by means of centrifugation or microcentrifugation, and the effects of such processing methods on total and fetal DNA in the plasma sample. *Id.* (citing Ex. 1003, 1608; Ex. 1002 ¶¶ 30–32, 39–41).

b) "isolating free fetal nucleic acid from the sample"

Petitioner argues that Chiu teaches claim 81's "isolating" step. Pet. 21–22. According to Petitioner, Chiu teaches "collecting in EDTA tubes blood samples from healthy pregnant women having a male fetus." *Id.* (citing Ex. 1003, 1608; Ex. 1002 ¶¶ 31, 41). Petitioner then cites Chiu's disclosures about further processing the blood samples by at least three distinct approaches to separate the plasma from cellular components in the sample: (1) centrifugation at 1,600*g*; (2) centrifugation followed by filtration of the separated plasma by a 0.2 μm filter; and (3) centrifugation followed by microcentrifugation of the separated plasma at 16,000*g*. *Id.* at 22 (citing Ex. 1003, 1608). In each case, Petitioner explains, Chiu then describes subsequent processing of the plasma and subjecting the samples to PCR for

[17] We need not determine whether the preamble is limiting to resolve this case because it is undisputed that Chiu teaches this subject matter.

detection of genes specific to fetal (*SRY* gene, male specific) DNA and total DNA (β-globin gene). *Id.* (citing Ex. 1003, 1609; Ex. 1002 ¶ 31).

> *c) "wherein said sample comprises an agent that inhibits lysis of cells, if cells are present, and wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor"*

For the two "wherein" clauses of claim 81, Petitioner contends that "Chiu is silent regarding an agent, besides EDTA, that inhibits cell lysis." Pet. 22.[18] According to Petitioner, "Chiu discloses that the samples should be processed to obtain plasma within two hours of collection, suggesting that EDTA tubes may not be sufficient for processing after two hours," and Chiu also "notes that apoptotic cells may be present in plasma from blood of pregnant women when the samples are processed only by [an initial] centrifugation." *Id.* at 22 (citing Ex. 1002 ¶ 41).

Petitioner turns to Bianchi for its alleged teaching of an "agent that inhibits lysis of cells" as recited in claim 81. According to Petitioner, Bianchi teaches a method where rare/target cells are permeabilized so target molecules within such cells can be labeled, and cells containing such targets can be identified in a sample. *Id.* at 21; *see* Ex. 1004, 2:14–30 (identifying intracytoplasmic molecules like proteins, and disclosing "fetal hemoglobin" as a preferred target). Petitioner cites Bianchi's teachings on permeabilizing the membrane of cells suspected of containing the target molecule by

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[18] Chiu includes EDTA as an anticoagulant to prepare plasma. Ex. 1003, 1608. Petitioner has not asserted in this case that EDTA is encompassed by the "agent" limitation of claim 81.

incubating the cells in a stepwise preparation of paraformaldehyde and alcohol at certain concentrations, times, and temperatures. Pet. 21 (citing Ex. 1004, 2:41–46, 3:36–44); *see also* Ex. 1004, 3:48–53 (disclosing that, after permeabilizing the cells, a labeling reagent (such as an antibody) is introduced to label the target molecule). Petitioner flags two features essential to Bianchi's method: the membrane of the cells must be made sufficiently permeable so that reagents that detectably label the target molecule can traverse the membrane and enter the cytoplasm; and the membrane must remain sufficiently intact so that substantially all the intracytoplasmic molecule and DNA remain within the target cell. *Id.* at 21–23 (citing Ex. 1004, 1:35–41, 3:25–35).

d) Motivation to Combine and Reasonable Expectation of Success

At a high level, Petitioner argues that a POSA would have been motivated to modify Chiu's method to include paraformaldehyde,[19] as disclosed in Bianchi. Pet. 22–23. The details of Petitioner's motivation-to-combine theory are discussed below.

Petitioner contends that it was known "that fetal DNA exists mainly in cell-free form and is not released significantly from dead or dying cells in the maternal circulation." Pet. 23. Petitioner contends that it was also known that "the amount of free maternal DNA was significantly increased [in samples] because of the liberation of DNA from maternal cells lysed

[19] Petitioner describes paraformaldehyde as a "polymerized form" of formaldehyde, and also as a "formaldehyde donor." *See* Pet. 17, 38, 42.

during clotting." *Id.*; Ex. 1003, 1607; Ex. 1023, 1; Ex. 1002 ¶¶ 24, 41–42. In support of Petitioner's arguments, Dr. Edwards opines that, when carrying out a method of processing maternal plasma for analysis of cffDNA (like Chiu), a POSA "would have been motivated to improve the detection of free fetal nucleic acids, for example, by increasing the percentage of fetal nucleic acids with respect to the total nucleic acids present by minimizing the introduction of any cellular DNA into the plasma sample." Ex. 1002 ¶ 24.

Petitioner contends that "Chiu discloses centrifugation and/or filtration as a means for reducing" maternal cells and maternal DNA in the assay background. Pet. 23–24 (citing Ex. 1002 ¶ 41). According to Petitioner, "Chiu also suggests that . . . processing in EDTA tubes may not be sufficient for processing after two hours." *Id.*; Ex. 1002 ¶ 41. Petitioner contends that a POSA would have been motivated "to try other means for reducing background, such as the inclusion of agents to inhibit the lysis of maternal cells." Pet. 24. Petitioner argues a POSA would have sought to determine "whether use of such agents" would work "as an alternative or in combination with EDTA, because it would eliminate or reduce apoptosis of cells during sample storage or processing (thereby eliminating or reducing background) or would potentially reduce the steps of preparing the sample for analysis (i.e., by eliminating a second centrifugation or filtration step)." Pet. 24 (citing Ex. 1002 ¶¶ 41–42); *see also id.* at 22–23 (arguing that "a [POSA] would have been motivated to try alternative or additional agents to

EDTA to reduce cell lysis in Chiu's methods" and "Bianchi provides such an alternative" with paraformaldehyde in an alleged "stabilization step").

Petitioner further argues that a POSA "would have had a reasonable expectation that use of paraformaldehyde in place of or in combination with EDTA with the centrifugation and/or filtration steps in Chiu's methods would have been successful for isolating and detecting fetal nucleic acids . . . as both techniques inhibit cell lysis and would have been expected to have reduced background." Pet. 24; Ex. 1002 ¶ 42.

Petitioner later expounds on its theory about the modification of Chiu's methods in view of Bianchi and why the POSA would allegedly have been motivated to make those changes. According to Petitioner, a POSA would, based on "general knowledge," have known "that blood samples, and particularly white blood cells, were stable for no more than twenty-four to forty-eight hours." Pet. Reply 17 (citing Ex. 1049,[20] 2:4–12). Moreover, Petitioner asserts, samples that required highly technical genetic analyses were "routinely shipped to . . . larger laboratories." *Id.* (citing Ex. 1049, 2:4–7). Thus, Petitioner contends, a POSA "would have looked to known methods for stabilizing blood samples and cells to enable shipment of samples to central laboratories." *Id.* (citing Ex. 1002 ¶¶ 21–22); *see also id.* at 18–19 (citing Ex. 1047 ¶¶ 15–16[21]). Allegedly, a POSA, "starting with

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[20] Exhibit 1049 is U.S. Patent No. 5,849,517, which issued December 15, 1998, and is assigned to Streck Laboratories, Inc. Ex. 1049, codes (45), (73). Exhibit 1049 was not cited or submitted with the Petition.

[21] This cited testimony of Dr. Edwards similarly refers to a need to ship samples to centralized labs for analysis because of the "relatively low total

Chiu would have looked to references, such as Bianchi . . . regarding stabilization of cells and general knowledge in the art regarding instability of blood samples." *Id.* at 17–18 ("[U]sing general knowledge in the art, [a POSA] would have selected the fixation step of Bianchi . . . to stabilize the cells.").[22]

Although Petitioner and its declarant, Dr. Edwards, acknowledged during trial that formaldehyde can potentially damage DNA, Petitioner

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amount of cell free fetal DNA and its relatively low percentage of total DNA in the blood and given the complexity of the genetic analysis." Ex. 1047 ¶ 15. Dr. Edwards further opines that "centrifuges and filtration are not available at all blood collection sites and . . . that such steps could decrease the amount of the sample and, thus, the amount of available fetal DNA" such that a POSA "would have been motivated to eliminate any steps that reduce the amount of fetal DNA." *Id.* Extending this underlying testimony to Chiu suggests that a POSA would modify the methods of Chiu to omit its on-site physical processing steps like microcentrifugation or filtration because, according to Dr. Edwards, those steps "could decrease the . . . amount of available fetal DNA." *Id.* Dr. Edwards provides no data or persuasive independent factual evidence, however, to support his opinion that *removing* the centrifugation or filtration steps of Chiu, which steps produced "cell-free" plasma samples with improved proportional recovery of detectable cffDNA (*see* Ex. 1003, 1609, 1613), would have been understood as providing means for greater total or proportional recovery of detectable cffDNA. Ex. 1047 ¶¶ 15–16; 37 C.F.R. § 42.65(a) (instructing that expert testimony that does not disclose underlying facts or data in support "is entitled to little or no weight").

[22] Patent Owner criticizes Petitioner's use of "newly-cited evidence and vague reliance on 'general knowledge' and 'creativity'" in the Reply as an improper and untimely attempt by Petitioner to fill holes in its theory. *See, e.g.*, PO Sur-Reply 3–5 ("Petitioner adds a new theory to support modifying Chiu based on 'general knowledge' and an alleged known need for

nevertheless asserts that "the use of formaldehyde as a nucleic acid fixative was fully characterized." Pet. Reply 19–20; Ex. 2299, 57:23–58:4 (testimony of Dr. Edwards agreeing that formaldehyde can damage DNA including cell-free DNA). Petitioner cites the Srinivasan[23] review article for its disclosure of "criteria recommended in the literature for use of formaldehyde as a tissue nucleic acid fixative." Pet. 19 (quoting Ex. 1051, 1965–1966) (disclosing, among other criteria, a "minimal prefixation time lag, < 2 hours" and total "duration of fixation (3 to 6 hours)"). Thus, Petitioner argues a POSA "would have been motivated to try and would have had a reasonable expectation of success in using formaldehyde or a formaldehyde donor to stabilize white blood cells in a maternal blood sample,"[24] and a POSA "would have understood that, although long durations or high concentrations of formaldehyde should be avoided,

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'shipment of samples to central laboratories.'"). We also have concerns about the evolution of Petitioner's obviousness challenge during this proceeding and about the timeliness of the submission of certain evidence in support of it. Petitioner's obviousness challenge is, in any event, unpersuasive for reasons we discuss below in our analysis.

[23] Mythily Srinivasan et al., *Review: Effect of Fixatives and Tissue Processing on the Content and Integrity of Nucleic Acids*, 161:6 American Journal of Pathology, 1961–1971 (Dec. 2002) (Ex. 1051 or "Srinivasan"). Petitioner filed Srinivasan with its Reply. Srinivasan is also of record as Exhibit 2150, which Patent Owner filed with its Response.

[24] As Petitioner's counsel confirmed, its theory is based on addition of formaldehyde or formaldehyde donors, not other agents. Tr. 22:24–23:17.

samples promptly processed and fixed yielded reliable nucleic acid analysis." *Id.* at 19–20 (citing Ex. 1047 ¶ 17).

At the oral hearing, Petitioner further clarified its motivation-to-combine story. Tr. 17:4–19:13. According to Petitioner, Chiu's approach addressed possible maternal cell lysis with a sophisticated "research laboratory method"—involving "rapid processing" and either filtration or microcentrifugation after an initial centrifugation to produce essentially cell-free plasma. *Id.* at 17:4–19:13. But, Petitioner contends, Chiu's approach did not address "a real-world problem" where blood is "collected at remote locations" and would, thus, need to be shipped to a central laboratory for processing and cell-free DNA analysis. *Id.* So, under its theory, Petitioner "envision[s] . . . that there would be a need to eliminate this immediate processing [of Chiu]," the blood-collection "tube could either contain the formaldehyde or formaldehyde donor . . . or it could be added to the blood after collection," and "[e]ither way, the point would be to then be able to transport it where, at a central location, it could be further processed because, naturally, it's going to have to be centrifuged to -- to achieve plasma." *Id.*

For the reasons above, Petitioner contends that claim 81 would have been obvious over Chiu and Bianchi.

2. Patent Owner's Arguments

Patent Owner raises several arguments why a POSA would not have been motivated to combine and modify the asserted prior art in the manner proposed with a reasonable expectation of success. PO Resp. 8–24; PO Sur-Reply 2–13. We focus our discussion on two of Patent Owner's arguments,

discussed in detail below, because we find such argument and related evidence decisive on this record.

First, Patent Owner argues that a POSA would not have combined Bianchi with Chiu because doing so would have been thought to permeabilize (add gaps or holes in) maternal cells in Chiu's blood sample, allowing DNA to escape and diluting the extracellular fraction of the sample, contrary to Chiu's goals. PO Resp. 14–19.

According to Patent Owner, Bianchi's cell-focused, cell-isolation protocols are fundamentally different from Chiu's methods for analyzing cell-free DNA in a maternal plasma sample. *Id.* at 14; PO Sur-Reply 7–8 (citing Ex. 2299, 152:21–155:3) (Dr. Edwards's testimony that Bianchi's paraformaldehyde is added to isolated mononuclear cells resuspended in a buffer—not blood samples that contain free nucleic acids)). Patent Owner contends that Bianchi is unconcerned with analysis of any extracellular blood component, much less cell-free DNA or cffDNA; as Patent Owner notes, the extracellular fraction in Bianchi is washed away and "simply discarded." PO Resp. 14–15 (citing Ex. 1004, 6:1–14); Ex. 1025, 574, 593; Ex. 2078 ¶¶ 95, 104). Also, Patent Owner contends, Bianchi is not concerned with reducing extracellular background from released DNA as evidenced by the fact that Bianchi's method "***permeabilizes cells***" so that materials can traverse the cell membrane. *Id.*

Patent Owner contends Bianchi's permeabilization approach runs contrary to the goal of reducing DNA background in the extracellular fraction of the sample, as in Chiu's method for analyzing cffDNA. PO Resp. 16–18. Although Bianchi endeavors to retain "substantially all" the

target analytes as well as the DNA within the target cells, Patent Owner contends that "even in the best scenario, its methods allow cellular contents—including DNA—to escape." *Id.* at 16 (citing Ex. 1004, 3:32–35; Ex. 2078 ¶ 97). Patent Owner cites Bianchi's disclosure that as much as 50% of the cellular molecules and DNA can escape notwithstanding Bianchi's preference for lower percentages down to about 1%. *Id.* (citing Ex. 1004, 1:42–48). Citing Dr. Van Ness's testimony, explaining the improbability that such lower DNA leakage rates would be expected in practice, Patent Owner contends that "[e]ven if the most preferred percentages were achieved, . . . a POSA would have appreciated that releasing 1% of cellular DNA in a sample" would add background DNA and "significantly dilute[] the cell-free fetal DNA Chiu seeks to detect." *Id.* at 16–18 (citing Ex. 2078 ¶¶ 98–103 (Dr. Van Ness's testimony explaining why this would frustrate Chiu's goals and method, greatly diminishing cffDNA concentrations); Ex. 2101, 107:21–108:17, 113:23–114:5 (Dr. Edwards's testimony that "any" leakage will "cause the fetal fraction to go down")).

Patent Owner also contends that Petitioner's theory "cherry-picks" paraformaldehyde from Bianchi and mischaracterizes the paraformaldehyde incubation as a "stabilization step." PO Resp. 18–19. According to Patent Owner, "Bianchi incubates with paraformaldehyde as part of the '***permeabilization*** method' (Ex. 1004, 3:36–43, Claim 8) and never describes paraformaldehyde as 'stabilizing' cells." *Id.* ("Bianchi provides no information about how that paraformaldehyde solution would affect cells without the permeabilizing reagents in Chiu's method."). In any case, Patent

Owner contends, even if a POSA would have understood that paraformaldehyde performs some cell-stabilizing function, Petitioner's own expert admitted on cross-examination that Bianchi's paraformaldehyde treatment "will cause gaps in the [cell] membrane." *Id.* (citing Ex. 2101, 102:15–103:20); *see also* Ex. 2101, 38:11–39:20 (testimony of Dr. Edwards that "there will probably be holes in the membrane when you add [formaldehyde] to a cell.").

Second, Patent Owner argues that a POSA would not have been motivated to use Bianchi's formaldehyde-based reagent in Chiu's method, and would not have reasonably expected success in doing so, because it was known that formaldehyde can damage DNA, and the cffDNA of Chiu is already a very scarce analyte. PO Resp. 20–24. As Patent Owner notes, "reasons to combine cannot be viewed in a vacuum apart from evidence suggesting reasons not to combine." *Id.* (quoting *Arctic Cat Inc. v. Bombardier Recreational Prods. Inc.*, 876 F.3d 1350, 1363 (Fed. Cir. 2017)).

According to Patent Owner, many sources reported on formaldehyde's detrimental effects on nucleic acids, undermining Petitioner's reasons for adding it to Chiu's method. *Id.*; Ex. 2078 ¶¶ 107–112. Patent Owner and Dr. Van Ness explain that interactions between formaldehyde and cell-free DNA, let alone cffDNA in plasma, were previously unknown (Ex. 2078 ¶ 109); but even as to DNA more generally, studies warned against using formaldehyde due to potential harm to nucleic acids. PO Resp. 21. The Srinivasan review article, for instance, states that "[a] method to overcome the problems of formaldehyde is to use an

alternative fixative that is better suited for the preservation of nucleic acids and proteins." Ex. 2150, 1966; *see also id.* at 1964–65 (noting "considerable evidence suggests that formaldehyde induces DNA degradation" and "formaldehyde initiates DNA denaturation . . . at the AT-rich regions of double-stranded DNA creating sites for chemical interaction").

According to Patent Owner, interactions between formaldehyde and DNA were known to arise from a number of detrimental effects. PO Resp. 20–22; *see, e.g.*, Ex. 2139, 945 ("Swenberg"). Swenberg, for example, discloses "single-stranded DNA breaks," "DNA-protein crosslinks," "sister chromatid exchanges," and "chromosome aberrations and mutations" resulting from the use of formaldehyde as a cellular fixative.[25] Such concerns about the potential for DNA damage, in the opinion of Dr. Van Ness, would have dissuaded the POSA from using formaldehyde (or paraformaldehyde) for a novel application with cffDNA detection and analysis. PO Resp. 21–22 (citing Ex. 2078 ¶¶ 109–110). As Patent Owner explains, the analysis of cffDNA was a completely new and developing field. *Id.* (explaining that, before the late 1990s and discovery of cffDNA

[25] Patent Owner cites several other references as evidencing the problems with formaldehyde's use, including damage to nucleic acids. Although such references do appear to report consistently on drawbacks with formaldehyde, many of those references post-date the filing date of the '277 patent by years. *See, e.g.*, PO Resp. 21–23 (citing Ex. 2155, published in 2005); Ex. 2047, published in 2018. For purposes of this decision, we do not rely on these references.

circulating in plasma, the plasma portion of the sample was "routinely discarded" (quoting Ex. 2038, 2:5–9)).

Continuing, Patent Owner argues, Petitioner has produced no evidence suggesting a use of formaldehyde in the context relevant here—for the preparation and analysis of cell-free nucleic acids. PO Sur-Reply 10–11. Patent Owner notes the many differences between cellular DNA and cell-free DNA, including that the latter is smaller, limited in quantity, and easily damaged. *Id.* at 12–13 (citing Ex. 2086 ¶ 4; Ex. 2299, 28:19–30:22 (testimony of Dr. Edwards that "the list is quite long in terms of the differences")).[26]

Patent Owner contends that Petitioner's theory that a POSA would have expected formaldehyde could be used safely and effectively with cell-free fetal DNA analysis does not hold up to scrutiny. PO Sur-Reply 10–12. Patent Owner cites Dr. Edwards's admission that formaldehyde can damage DNA, including cell-free DNA. *Id.* at 10 (citing Ex. 2299, 57:23–58:4). Patent Owner argues that Petitioner's citation to Srinivasan does not fill the

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[26] Patent Owner also points to a recent Board decision as recognizing the differences between cellular and cell-free DNA, and whether fixatives are interchangeable for use with them. PO Sur-Reply 12–13 (citing *Ex Parte Fernando*, Appeal No. 2021-003268, 2022 WL 855866, at *12 (PTAB Mar. 21, 2022) (crediting the argument that "stabilization methods suitable for stabilization of blood samples do[] not necessarily translate into suitable stabilization methods for cell-free fetal nucleic acids")). That case relates to an appeal of the Office's rejection of claims in a January 19, 2010, patent application owned by third-party Streck, Inc. Ex. 2298, 1–2. That decision was based on specific arguments raised in that case and the record developed there; it does not control the outcome in this IPR.

holes in Petitioner's theory because Srinivasan does not discuss cell-free DNA at all; if anything, Srinivasan "urges avoiding" formaldehyde even for "***tissue*** [(i.e., cellular)] ***nucleic acids***." *Id.* (citing Ex. 1051, 1964–1966; Ex. 2299, 41:17–25). On whether a POSA would be unconcerned with formaldehyde's problems or just "tailor" conditions (time of fixation, concentrations, etc.) to address them (*see, e.g.*, Pet. Reply 19), Patent Owner contends that notion is at odds with testimony of Petitioner's own expert in related litigation. *Id.* at 9–10 (citing Ex. 2300 ¶ 390 (testimony of Dr. Larry Dumont about formaldehyde degrading nucleic acids and inhibiting PCR, even at low concentrations)). Moreover, Patent Owner contends, Petitioner's invocation of Srinivasan's "criteria" for using formaldehyde (e.g., limiting exposure to "3 to 6 hours" to reduce DNA damage) conflicts with Petitioner's proffered motivation to stabilize blood samples for longer than 24–48 hours, so that samples may be shipped away to a central lab for centrifugation and cffDNA analysis. *Id.* at 11. According to Patent Owner, Petitioner never clarifies how the POSA would apply such "criteria" to stabilize blood for the long durations of formaldehyde exposure contemplated by Petitioner's modified Chiu/Bianchi method. *Id.*

For at least the above reasons, Patent Owner contends Petitioner has not met its burden to prove, by a preponderance of the evidence, that claim 81 is unpatentable as obvious over Chiu and Bianchi.

3. Analysis

On this record, we agree with Patent Owner that Petitioner has not carried its burden to establish that a POSA would have been motivated to add paraformaldehyde to the modified blood sampling and cffDNA

detection method of Chiu.[27] More specifically, we find on this record that a POSA would have been dissuaded from adding Bianchi's paraformaldehyde because the POSA would have expected Bianchi's paraformaldehyde to create gaps in the cell membranes, providing a means for maternal DNA to escape into the sample. We also find on this record that a POSA would have been dissuaded from adding paraformaldehyde to Chiu's methods because formaldehyde was known to damage nucleic acids.[28] Patent Owner's reasoning and evidence on those issues, separately and cumulatively, outweigh Petitioner's comparatively weak showing on whether a POSA would have combined the art in the manner proposed. We discuss in greater detail below.

We begin with the issue that paraformaldehyde contributes to permeabilization of the cellular membrane. We credit Dr. Van Ness's opinion that adopting Bianchi's approach to treating cells with paraformaldehyde creates a means for cellular DNA to escape. Ex. 2078

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[27] *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1327 (Fed. Cir. 2016) ("Whether an ordinarily skilled artisan would have been motivated to modify the teachings of a reference is a question of fact.") (citations omitted); *Alza Corp. v. Mylan Labs., Inc.*, 464 F.3d 1286, 1289 (Fed. Cir. 2006) ("The presence or absence of a motivation to combine references in an obviousness determination is a pure question of fact").

[28] When asked whether he considered "any differences between paraformaldehyde and formaldehyde that might be relevant to DNA damage in forming your opinions," Dr. Edwards testified "[t]here should be no differences" and that "[i]n this field, these terms are often used interchangeably." Ex. 2299, 69:3–20. We find, therefore, that the issues, including DNA damage, with formaldehyde would have been understood to also apply to paraformaldehyde on this record.

¶¶ 96–98; Ex. 2101, 106:4–19 (testimony of Dr. Edwards agreeing that "DNA is one thing that can escape a cell when you permeabilize it"). Although Bianchi discloses a desire to keep "substantially all" of the target molecules inside the cells, by Bianchi's own definition, "substantially all" includes leakage of up to 50% of the target molecules and cellular DNA. Ex. 1004, 1:42–49; Ex. 2078 ¶¶ 99–101. We find that increasing potential leakage of DNA from the maternal cells runs counter to Chiu's objectives. As Dr. Edwards concedes, "DNA leaking out of cells" is something "Chiu tells us you do not want it to happen." Ex. 2101, 107, 21–25.

Petitioner contends that its modification of Chiu relies only on Bianchi's alleged "stabilization step." Pet. Reply 23; *see also id.* at 18 (arguing that "Bianchi . . . included a permeabilization step separate from the fixation step") (citing Ex. 1045, 171:3–11 (testimony of Dr. Van Ness that he has performed assays with permeabilization and fixation steps)). We agree with Patent Owner, however, that Bianchi does not describe using paraformaldehyde in any separate stabilization or fixing step. PO Sur-Reply 7–8. Instead, Bianchi teaches a "permeabilization method" where the cell preparation is first incubated in paraformaldehyde and "***then*** is incubated ***permeabilized***" in a solution containing alcohol. Ex. 1004, 3:36–44 (emphasis added); Ex. 2078 ¶¶ 105–106 (testimony of Dr. Van Ness discussing Bianchi's permeabilization process). This suggests the paraformaldehyde incubation contributes, to at least some degree, to permeabilizing cells. And, even if the POSA would understand that paraformaldehyde provides some cell-stabilization function, Dr. Edwards admits that adding formaldehyde or paraformaldehyde will likely create

"holes" and "gaps" in the cell membrane—even as the membrane becomes more rigid. Ex. 2101, 38:11–39:20 (testifying "there will probably be holes in the membrane when you add it to a cell" because "[i]t's a pretty harsh treatment on cells" and "my guess is that it would impact the entire fluidity of the membrane"), 103:15–20 (testifying that, as in Bianchi's method, "using a fixative to basically probably rigidify, if you will, the cell, which will cause gaps in the membrane in some ways probably").

Returning to DNA leakage, we acknowledge that Bianchi prefers that greater amounts of DNA stay in the cells. *See* Ex. 1004, 1:42–48 (disclosing a range: a lower bound of "preferably 50%" and an upper bound of "more preferably 95" and "most preferably 99% or greater" of "the DNA of the cell remain in the cell"). But we credit Dr. Van Ness's unrebutted testimony that Bianchi does not disclose how the POSA could adjust its techniques so the cells may leak at greater or lesser rates, and that a POSA would understand the probability of obtaining a leakage rate of 1% is low. Ex. 2078 ¶¶ 100–102. Assuming the improbable occurred, and only about 1% of the DNA escaped, Dr. Van Ness testifies persuasively that a "POSA would realize that releasing 1% of cellular DNA in a sample of Chiu would have a negative effect on Chiu's fetal cell-free DNA analysis." *Id.* ¶¶ 102–103 (citing Dr. Edwards's testimony (Ex. 2101, 113:23–114:5): Q "[D]id you consider what effect leaking 1 percent of the cellular DNA in samples in Chiu would have on Chiu's free fetal DNA percentages? A[:] Well, leaking any is going to cause the fetal fraction to go down.")). Indeed, Dr. Edwards admits that "any DNA escaping from cells in Chiu can lower the free fetal fraction in a sample"—contrary to Chiu's goals. Ex. 2101, 107:21–108:11.

Petitioner vaguely invokes "general knowledge" in response, and contends that Bianchi's "fixation" step is the only important disclosure (discussed above). Pet. Reply 15–18.[29] Petitioner does not, however, provide persuasive argument or evidence to explain why creating holes in the cell membranes—possibly releasing 1% or more of the maternal DNA—would have been seen by the POSA as acceptable in the modified method of Chiu.

On this record, we find the DNA leakage that would have been expected from adding paraformaldehyde undermines Petitioner's position that a POSA would have been motivated to modify Chiu as proposed.

We also agree with Patent Owner that Petitioner has not established that the POSA would have been motivated to add paraformaldehyde to the modified blood sampling and cffDNA-detection method of Chiu because the current record supports a connection between formaldehyde's use and DNA damage. On balance, we are persuaded on this record that the POSA would have had significant, unresolved concerns with introducing formaldehyde,

[29] Dr. Edwards's Supplemental Declaration devotes only four paragraphs to a response on the issue of motivation to combine (covering all grounds). Ex. 1047 ¶¶ 14–17. Most of that testimony relates to a purported need to stabilize and ship samples to central labs and a known use of formaldehyde as a *cellular* fixative (with no apparent suggested or reported use with cell-free DNA). *Id.* We have considered this testimony, but find that it provides no direct or persuasive response to Dr. Van Ness's testimony, which we credit on this record, about the concerns with leaking even small proportions of maternal DNA in a cffDNA detection method under Petitioner's modification of the art. Ex. 2078 ¶¶ 99–106.

and its potential to adversely affect cffDNA in the modified method of Chiu, undermining Petitioner's challenge.

Cellular DNA and cell-free DNA are not the same thing. Dr. Edwards confirms this—explaining that they are "quite different," and that the list of differences is "quite long." Ex. 2299, 29:7–30:22. It is not simply the location of the DNA as intracellular versus extracellular; there are also structural and biochemical differences. For example, cell-free DNA are short fragments compared to cellular DNA which is typically long, genomic DNA. *Id.* (noting the scale of the size difference with cell-free DNA typically "very small, on the order of a hundred base pairs" whereas cellular DNA may be "hundreds of millions of bases long"). There are also differences in methylation patterns and binding proteins—cellular DNA is predominantly intact, wrapped around proteins forming chromatin (a DNA/protein complex). *Id.*; *see also id.* at 32:11–16 (cell-free DNA is comparatively protein free (the DNA/protein complex form is eliminated)); Ex. 2101, 101:2–12. Patent Owner cites evidence on additional differences that are alleged to create obstacles to cffDNA recovery, including that it is available in limited quantities, can be diluted due to lysis, and is "easily damaged." Ex. 2086 (Hunsley Decl.) ¶ 4. Dr. Edwards agreed that cffDNA is small, available in limited quantities, and diluted by lysis, and "agree[d] it can be damaged" but demurred, "I don't know if I agree with the easily part." Ex. 2299, 158:19–161:22.

A key question presented in this case is whether a POSA would have been concerned with formaldehyde's potential effects on DNA, and cell-free fetal DNA in particular. Petitioner argues that a POSA would have been

motivated to try "other means for reducing [maternal DNA] background," including adding paraformaldehyde to the method of Chiu, but the Petition does little to address whether that addition would have been expected to adversely affect cell-free DNA, much less cffDNA.[30] Pet. 23–24. Bianchi does not speak specifically to that issue because, as Dr. Edwards admits, the DNA mentioned in Bianchi is "cellular" DNA and whatever DNA escapes the cells is just washed away. Ex. 2299, 59:16–19; Ex. 1004, 6:1–14); Ex. 2078 ¶¶ 95, 104. And, although Dr. Edwards concedes that it was known that formaldehyde can damage DNA—and the record includes evidence cautioning against the use of formaldehyde-containing compounds, even for applications that involved *cellular* DNA—Dr. Edwards admitted that, for his initial analysis, he "didn't consider whether the negative impacts of formaldehyde might have led someone in 2002 to pick a different chemical instead." Ex. 2101, 68:3–17; Ex. 2299, 57:23–58:4 ("[Y]ou don't dispute that formaldehyde has the potential to damage DNA including cell-free DNA, right? A[:] That is correct, It has the potential."). Petitioner's

[30] Petitioner initially suggested that paraformaldehyde might "replace" EDTA in Chiu's samples and method. Pet. 23–24. It is unclear why a POSA would have thought that paraformaldehyde might replace EDTA, the anticoagulant in Chiu. Petitioner directs us to no evidence showing paraformaldehyde or formaldehyde were considered alternatives to an anticoagulant, or suggesting plasma samples (as in Chiu) could be prepared without an anticoagulant.

evidence in support of its alleged motivation to add paraformaldehyde is deficient on this record.

Patent Owner responded with evidence that a POSA would have been concerned about the detrimental effects of formaldehyde on nucleic acids. Srinivasan, for example, notes that attempts to extract usable DNA from formaldehyde fixed tissues have only been "variably successful," explaining that "considerable evidence suggests that formaldehyde induces DNA degradation" with "few" studies reporting yield of high-molecular weight DNA. Ex. 2150, 1964 (disclosing "formaldehyde initiates DNA denaturation" and a high frequency of "sequence alteration"). Srinivasan further indicates that "the problems of formaldehyde" might be overcome by "use [of] an alternative fixative that is better suited for the preservation of nucleic acids." *Id.* at 1966. Patent Owner also cites Swenberg, as it reports on the problems with using formaldehyde, even with cellular applications and cellular DNA. PO Resp. 20–21 (citing Ex. 2139, 1 (identifying "DNA breaks" among other problems)). We credit Dr. Van Ness's opinion that such disclosures in the literature would have dissuaded a POSA from using formaldehyde or paraformaldehyde in Chiu's modified method. Ex. 2078 ¶¶ 109–110. That is especially so here, where we have a dearth of evidence suggesting formaldehyde's use in a sample where *cell-free* DNA is the analyte, and no sufficient, persuasive evidence or technical reasoning to explain why a POSA would not have been concerned with potential damage to the cffDNA.

In its Reply, Petitioner ignores Swenberg and embraces Srinivasan's alleged "criteria" for "the use of formaldehyde as a nucleic acid fixative."

Pet. Reply 19 (citing Ex. 1051, 1965). Insofar as Petitioner suggests that Srinivasan discloses criteria for using formaldehyde to fix *free* nucleic acids, Petitioner is incorrect. It does not. The portion of Srinivasan cited by Petitioner relates to use as a "***tissue*** nucleic acid fixative"—in other words, *cellular* nucleic acids. Ex. 1051, 1965 (emphasis added). Petitioner's expert admits that Srinivasan does not discuss cell-free DNA or cffDNA. Ex. 2299, 41:15–25 ("Q[:] Is it your understanding that the Srivastan [sic] paper discusses cell-free DNA at all? A[:] ***No just DNA damage due to fixation***.") (emphasis added)). On balance here, we find that Srinivasan is not specific to cell-free DNA and otherwise discourages formaldehyde's use due to potential harms to DNA more generally.

Petitioner also never explains how the cited "criteria" in Srinivasan align with Petitioner's proffered modification to Chiu's method. As discussed above, Petitioner purports to modify Chiu's method so that the cells are stabilized with paraformaldehyde for relatively long time periods—more than 24 to 48 hours to enable shipping off-site to a central lab. *See supra* Section II(E)(1)(d). That theory stands in tension with Srinivasan's disclosure that duration of tissue fixation should not exceed 3–6 hours. Ex. 1051, 1965; *see* Pet. Reply 20 (arguing a POSA would know that "***long durations*** or high concentrations of formaldehyde ***should be avoided***, [but] samples promptly processed and fixed yielded reliable nucleic acid analysis" (citing Ex. 1047 ¶ 17 (citing Srinivasan's 3–6 hour fixation time)) (emphasis added). Petitioner has not reconciled Srinivasan's disclosures with Petitioner's theory for modifying Chiu and Bianchi, which theory presumes

a long and sustained exposure of the maternal blood sample to paraformaldehyde.

When asked at the oral hearing how its theory was compatible with criteria indicating exposure of no more than 3–6 hours, Petitioner responded that there was an alleged "need for formaldehyde donors and quenchers, which is actually what's been adopted in the industry." Tr. 20:22–21:14 (identifying "Cyto-Chex and Rao reference," as well as the '517 patent (Ex. 1049)). If Petitioner wanted to assert a combination based on Chiu and Streck's '517 patent, it could have made that argument in the Petition. It did not. Petitioner also never proposed as part of its theory in any paper that a POSA would have further added "quenchers" to its combination of Chiu and Bianchi. The oral hearing is too late. (At best, Petitioner's response at oral argument citing Cyto-Chex and Rao relates to its ground based on the combination of Chiu and Rao, which we address below.) When pressed on the fact that Petitioner's theory requires cffDNA undergo "sustained exposure to whatever the stabilizer is," counsel shifted again, noting "different variables . . . that you could use, you could decrease the concentration of formaldehyde." *Id.* at 22:7–19. This too is not explained sufficiently in Petitioner's papers. It is of the "utmost importance" that a petitioner identify with particularity its theories and supporting evidence in the petition itself. *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1369 (Fed. Cir. 2016) (explaining that such particularity is required by statute under 35 U.S.C. § 312(a)(3)).

Finally, we agree with Patent Owner that, inasmuch as Petitioner is suggesting a POSA might simply "tailor" the processing conditions for using

formaldehyde effectively, Petitioner's argument fails. PO Sur-Reply 9–10. As Patent Owner explains persuasively, Petitioner leaves it "entirely unclear" what those specific conditions are and how they would be modified to render formaldehyde safe for use with Chiu's cffDNA. *Id.* Petitioner's suggestion is also at odds with testimony from its expert in the parallel litigation. *Id.* at 9 (citing Ex. 2300). Indeed, in that proceeding, Dr. Larry Dumont testified that "formaldehyde . . . ha[s] been demonstrated to degrade nucleic acids," that "low concentrations of formaldehyde . . . significantly inhibit[] (or eliminates altogether) the ability to successfully perform PCR amplification on DNA," and that "literature suggests that these 'agents' would not be compatible with standard methods used to detect and analyze DNA.")). Ex. 2300 ¶ 390.[31] Petitioner has not shown sufficiently, on this record, that a POSA would have understood that paraformaldehyde could be used effectively with Chiu's cffDNA. The evidence presented by Patent Owner undermines Petitioner's assertion that a POSA would have been motivated to modify Chiu as proposed.

Altogether, considering the argument and evidence presented through trial, Petitioner does not persuade us that a POSA would have been

[31] It appears Dr. Dumont's testimony related, in particular, to whether certain of the '277 patent's claims were enabled. *See, e.g.*, Ex. 2300 ¶ 369.

motivated to combine Chiu and Bianchi in the manner proposed to arrive at the subject matter of claim 81.[32]

4. Dependent claims 82–91, 94–96 and 133

Claims 82–91, 94–96, and 133 all depend from claim 81. For these dependent claims, Patent Owner raises the same arguments as addressed above. PO Resp. 8 (argument for "All Claims"). Petitioner's challenge to the dependent claims relies on its claim 81 analysis (*see, e.g.*, Pet. 25), and Petitioner does not argue or show that its challenge to the dependent claims makes up for deficiencies we have noted above. Pet. Reply 13–20 (same responsive argument for all claims); *see supra* Section II(E)(3). Thus, we conclude that Petitioner has not proved that claims 82–91, 94–96, and 133 are unpatentable based on the combination of Chiu and Bianchi. *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992) ("[D]ependent claims are nonobvious if the independent claims from which they depend are nonobvious.").

F. Obviousness over Chiu, Bianchi, and Granger

Petitioner asserts that dependent claims 92 and 93 would have been obvious over Chiu, Bianchi, and Granger. Pet. 36–39. Petitioner relies on its showing for claims 81, 90, and 91, from which claims 92 and 93 depend.

[32] We need not reach Patent Owner's argument on secondary considerations of nonobviousness here. *See Hamilton Beach Brands, Inc. v. f'real Foods, LLC*, 908 F.3d 1328, 1343 (Fed. Cir. 2018) (holding that there is no need to reach objective indicia of nonobviousness where the petitioner has not made a showing necessary to prevail on threshold obviousness issues).

Id. at 31–32, 37.[33] Petitioner cites Granger's disclosure on stabilizing leucocytes in an aldehyde-containing solution, preferably paraformaldehyde dissolved at a concentration from 0.1% to 0.5% w/v. *Id.* at 37. Patent Owner raises the same arguments as discussed above for the ground based on Chiu, Bianchi, and Granger. PO Resp. 7–24 (arguing grounds 1 and 2 together for all claims). Petitioner does not argue or show that its challenge to these dependent claims makes up for deficiencies we have addressed above. Pet. Reply 13–20 (same responsive argument for all claims). Thus, we conclude that Petitioner has not proved that claims 92 and 93 would have been unpatentable based on the combination of Chiu, Bianchi, and Granger. *See supra* Section II(E)(3)–(4).

G. Obviousness over Chiu and Rao

Petitioner asserts that claims 81–96, and 133 would have been obvious over Chiu and Rao. Pet. 39–57; *see id.* at 39–44 (claim 81). Chiu and Rao are discussed above. *See* Section II(D)(1), (3). Petitioner's theory on Chiu and Rao is substantially similar to the Chiu/Bianchi challenge. Petitioner relies on the same teachings in Chiu as disclosing methods for isolating and analyzing cffDNA from maternal plasma. *Id.* at 40–41. As with the grounds above, Petitioner contends that Chiu is "silent" on

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[33] The Petition urges, for these two claims, that a POSA would have "explore[d] a range of formalin and paraformaldehyde concentrations," and would have been motivated to use a low concentration due to formaldehyde's "noxious nature," but such argument fails to address persuasively the fact that formaldehyde was known to damage DNA itself, as discussed above. Pet. 38–39.

disclosing a lysis-inhibiting "agent" as claimed and, thus, Petitioner turns to Rao in a similar way to Bianchi. *Id.* at 41–42. Petitioner contends that a POSA "would have been motivated to try alternative or additional agents to EDTA to reduce cell lysis in Chiu's methods" and, like it did with Bianchi, Petitioner contends that Rao discloses "such an alternative" by teaching cell-stabilizing compounds that may stabilize rare cells. *Id.* (citing Ex. 1005, Abstr.; Ex. 1002 ¶¶ 34, 53). According to Petitioner, Rao identifies commercial stabilizers like "Cyto-Chex™ from Streck Laboratories," a "formaldehyde donor" like "imidazolidinyl urea . . . [or] paraformaldehyde," or an "aldehyde" like "formaldehyde." *Id.*

Petitioner's motivation to combine and reasonable expectation of success positions largely mirror its argument on Chiu/Bianchi. *Id.* at 42–44. Petitioner contends a POSA would have been motivated to try other means to reduce maternal DNA background, including adding agents to inhibit cell lysis, and to determine whether such agents may provide advantages over Chiu's use of EDTA with filtration or microcentrifugation steps. *Id.* (citing Ex. 1002 ¶ 53); *see also id.* (arguing a POSA would have been motivated to add agents "such as the agents described in Rao" to reduce apoptosis during storage and possibly simplify Chiu's processing steps "(i.e., by eliminating a second centrifugation or filtration step)"). According to Petitioner, a POSA "would have had a reasonable expectation that use of an aldehyde, or other cell lysis inhibitor disclosed in Rao, in place of or in addition to the EDTA used with the centrifugation and/or filtration steps in Chiu's methods, would have been successful for detecting fetal nucleic acids in a sample as both

techniques inhibit cell lysis and would have been expected to reduce assay background created by lysed maternal cells." *Id.*

Petitioner expounds on its motivation theory as discussed above (*supra* Section II(E)(1)(d)), and, although Petitioner's assertions in the Petition, noted immediately above, refer somewhat vaguely to adding Rao's alleged "agents" to Chiu, Petitioner confirms that its theory is based on the addition of formaldehyde or formaldehyde donors, not other agents in Rao. Tr. 22:24–23:17.[34]

Based on Petitioner's overlapping argument, Patent Owner's argument against the combination of Chiu and Rao tracks its argument about Chiu and Bianchi, discussed above. PO Resp. 37–47.[35] For example, Patent Owner contends that Rao's cell-detection approach adds agents that "creat[e]

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[34] Petitioner has argued that Cyto-Chex blood collection tubes "contain[] EDTA and a formaldehyde donor (imidazoli[di]nyl urea)." Pet. Reply 12 (citing Ex. 1050, 9:12–17); *see also* Ex. 2086 ¶ 6 (testimony of Brad Hunsley, Streck Inc.'s Director of Research & Development that Streck's Cyto-Chex blood-collection tubes have been available for sale since about 2003, and that such products are the subject of US Patent Application No. 10/605,669 (which appears to be the application filed October 16, 2003, that issued April 6, 2021, as US 10,966,421 (Ex. 1050)). For purposes of this decision, we will treat that characterization of Cyto-Chex by Petitioner as accurate and that it includes a formaldehyde donor. We note, however, that Rao does not explicitly list Cyto-Chex's components.

[35] Patent Owner disputed the prior-art status of Rao for dependent claims 90, 91, and 133. PO Sur-Reply 13–18. Petitioner, conversely, alleged that such claims do not have an effective date before August 29, 2003, and, if they did, Rao would still be prior art based on Rao's provisional application filing date in August 2001. Pet Reply 3–13. We need not decide this issue to resolve the case because, even if Rao is prior art for all claims, Petitioner's challenge based on Chiu and Rao fails for other reasons, as discussed herein.

more permeable cells that allow particles to travel through their membrane, thus increasing the potential release of DNA from cells." *Id.* at 43–44 (citing Ex. 1005, 12:26–30, 13:16–23; Ex. 2078 ¶ 136 (testimony of Dr. Van Ness that applying Rao's teachings would increase the risk of releasing DNA and increasing DNA background)). Patent Owner also argues, like it did with Bianchi, that Rao is unrelated to detection or analysis of cell-free DNA; to the extent DNA is mentioned in Rao, Patent Owner argues Rao describes concerns with formaldehyde, including its negative impacts on DNA. *Id.* at 44–45; Ex. 2078 ¶¶ 138 (Dr. Van Ness explaining, *inter alia*, that Rao discards the extracellular fraction of its samples), 139 (opining, e.g., that Rao discloses that formaldehyde released from formaldehyde donors was known to "irreversibly cross link[] nucleic acids," citing Ex. 1005, 4:8–11); PO Sur-Reply 19–20 (citing Ex. 2299, 146:11–147:21 (admissions of Dr. Edwards that Rao does not analyze plasma or DNA, or determine whether its stabilizers had caused harm to DNA or rendered it unsuitable for assay methods like PCR)).

Continuing, Patent Owner argues, "Petitioner's focus on formaldehyde for its Chiu/Rao combination suffers from the same deficiency as its Chiu/Bianchi combinations." PO Resp. 45. Like it argued above, Patent Owner contends Petitioner fails to address adequately the dangers of formaldehyde and its known potential to damage nucleic acid products. *Id.* at 45–46 (citing Ex. 2078 ¶¶ 140–146; cross-referencing its argument and evidence contra the Chiu/Bianchi combination). According to Patent Owner, as with publications such as Srinivasan, "Rao itself would have dissuaded a POSA from using formaldehyde" because of its noted problems

and deficiencies. *Id.* (citing, e.g., Ex. 2150, 1966 (recommending alternative fixatives even for cellular applications); Ex. 1005, 4:8–13 (Rao disclosing that formaldehyde from donors is reported to react with nucleic acid bases and irreversibly cross-link DNA); Ex. 2101, 150:4–16 (testimony of Dr. Edwards that formaldehyde will "cause cross-links to DNA")).[36]

Rather than substantially repeat the analysis and evidence discussed above (*supra* Section II(E)(3)-(4) & II(F)), we cross-reference and adopt that discussion here. In summary, we find that Petitioner has not proved by a preponderance of the evidence that a POSA would have added formaldehyde or a formaldehyde donor from Rao to the modified method of Chiu. As explained above, we find on this record that a POSA would have been discouraged from adding reagents that would create holes in the cells because that provides route for DNA to escape and potentially adds undesired maternal background DNA. Dr. Edwards admits that formaldehyde will "create holes in membranes" and characterizes formaldehyde as "a pretty harsh treatment on cells . . . when you do this." Ex. 2101, 39:8–15; *see also id.* at 107:22–25 (testifying that DNA leaking from cells is something "Chiu tells us you do not want it to happen"); Ex. 2078 ¶ 136 (testifying that Rao's treatment of cells makes them more permeable).

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[36] Petitioner's and Patent Owner's argument in the respective Reply and Sur-Reply about a motivation to combine Chiu and Rao are also substantially the same as argued for Chiu/Bianchi. Pet. Reply 14–20 (arguing all grounds together); PO Sur-Reply 19–20 (cross-referencing Chiu/Bianchi argument).

Even if the DNA leakage issue would not have caused the POSA to turn away from introducing formaldehyde, we credit Patent Owner's rebuttal evidence showing that formaldehyde was known to cause problems, including DNA breaks and degradation, even relative to *cellular* applications and *cellular* DNA (as opposed to cell-free DNA, where formaldehyde's effects were previously unknown and unreported). *See, e.g.*, Ex. 2150, 1964–1966. We also credit Dr. Van Ness's opinion that concerns with formaldehyde would, on balance, have discouraged the POSA from using it in the context of cffDNA analysis. Ex. 2078 ¶¶ 140–146. Such evidence belies Petitioner's assertion that a POSA would have been motivated to add formaldehyde or formaldehyde-releasing donor compounds to the method of Chiu.

Even Rao discloses that "[f]ormaldehyde released from these so-called formaldehyde donors has been reported to react with nucleic acid bases, particularly adenine, to reversibly form hydroxymethylol derivatives and methylene bridges thereby ***irreversibly crosslinking*** nucleic acids." Ex. 1005, 4–11 (emphasis added); Ex. 2078 ¶ 143 (testimony of Dr. Van Ness that such cross-linking would be detrimental for nucleic-acid applications). Dr. Edwards admits that adding formaldehyde "could induce cross-links, and they need to be reversed" in order to amplify cffDNA for analysis. Ex. 2101, 150:3–151:2 (admitting formaldehyde's addition will "cause cross-links to DNA").[37] How the "irreversible" DNA crosslinks of

[37] *See* Ex. 2101, 72:17–22 ("Q[:] But could cross-links on DNA affect one's ability to amplify or detect the DNA? A[:] Well, one would need to reverse

the sort described in Rao would be reversed, or why that would (or would not) be a concern, is left unaddressed by Petitioner. Pet. Reply 14–20 (not addressing this argument); Ex. 2078 ¶¶ 14–17 (rebuttal testimony of Dr. Edwards on motivation-to-combine issues, not addressing Rao's disclosure of irreversible crosslinking of DNA by formaldehyde donors).

Petitioner identified Cyto-Chex as a stabilizer disclosed in Rao, yet Cyto-Chex still involves a formaldehyde donor. Pet Reply 12 (asserting that Cyto-Chex includes the formaldehyde donor imidazolidinyl urea); *see supra* n.34. There is no dispute on this record that formaldehyde donors will still release formaldehyde. *See, e.g.*, Ex. 1005, 3:26–4:20 (describing the release of formaldehyde by such donors). And, as such, we find here that similar concerns to those above would have discouraged the use of Cyto-Chex in a wholly new way involving cffDNA.

During the oral hearing, Petitioner suggested for the first time that a POSA may have combined "formaldehyde donors and quenchers" to avoid damage from sustained exposure of cell-free DNA to formaldehyde. Tr. 20:22–21:14 (asserting that such combination is "actually what's been adopted in the industry" and "that really gets us back to the Cyto-Chex and Rao reference," and further "Cyto-Chex, according to the '517 patent, can include a formaldehyde donor and a formaldehyde quencher"). As noted above, Petitioner never asserted in any pre-hearing paper that "quenchers" would be added in its combined prior art. Moreover, Streck's '517 patent

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the cross-links, and then they should -- one should be able to amplify or detect the DNA.").

(Ex. 1049) was not cited in the Petition. And, although it is cited in Petitioner's Reply, Petitioner did not identify alleged teachings about "quenchers" to argue that a POSA would have selected such compounds for further addition in its Chiu and Rao combination.[38] Pet. Reply 17–20. On this record, we find that Petitioner forfeited the argument that the POSA would have added formaldehyde-quenching compounds to its combination based on Chiu and Rao. Paper 44 (Order setting oral hearing), 4 (citing, e.g., *Dell Inc. v. Acceleron, LLC*, 884 F.3d 1364, 1369 (Fed. Cir. 2018) (holding that the Board is obligated under its own regulations to dismiss untimely argument "raised for the first time during oral argument")).

Even if we were to consider Petitioner's untimely argument, Petitioner makes no persuasive showing that Streck's '517 patent relates to, or suggests any use of, formaldehyde donors with cell-free DNA or cffDNA. And, absent evidence tying the alleged industry practices to what a POSA would have done at the time the '277 patent was filed, Petitioner's assertion that

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[38] We recognize that Rao lists the '517 patent among at least seven other Streck patents identified as disclosing stabilizing agents. Ex. 1005, 3:33–4:3 (listing "US 5,849,517" among others). But it is not apparent, and Petitioner never argued, that the '517 patent is incorporated by reference into Rao. Assuming arguendo that the '517 patent is in some manner related to Cyto-Chex, the mere disclosure of the '517 patent in Rao does little to further Petitioner's case, especially absent timely argument and evidence about the alleged obviousness of adding quenching compounds. In any event, there is evidence of record that the '517 patent is not related to Cyto-Chex. Streck's Director of R&D testified that another product called "Streck Cell Preserve" is the subject of the '517 patent. Ex. 2086 ¶ 6; *see also id.* ¶ 7 (testifying that Cyto-Chex BCTs (blood collection tubes) are the subject of another patent application (which is not identified in Rao)).

what the industry has actually done is use formaldehyde donors and quencher compounds, even if true, does not weigh in favor of a determination of obviousness on this record. Moreover, the record here suggests that any industry adoption of such compounds for use with cffDNA analysis occurred only *several years after* the filing (in 2003) and publication (in 2004) of the application that matured into the challenged '277 patent. Ex. 1001, codes (22), (65)); Ex. 2109, cover page (U.S. Patent 9,926,590, assigned to Streck, Inc., titled "Devices and Compositions for Preservation of Cell-Free Nucleic Acids" claiming priority through many applications, the earliest of which are in 2009 and 2010), 13:20–45 (claim 1); Ex. 2108, 1 (Streck website listing U.S. Patent 9,926,590 as covering its "Cell-Free DNA BCT®"); Ex. 2086 ¶¶ 6–8 (testimony of Mr. Hunsley that, prior to 2010, "there is no literature to suggest use of Streck Cell Preserve or Cyto-Chex® BCT for use in fetal DNA recovery"). PO Sur-Reply 22–23 (citing Exs. 2108, 2109, 2086).

For the reasons above, we conclude that Petitioner has not proved by a preponderance of the evidence on this record that claims 81–96, and 133 are unpatentable as obvious over Chiu and Rao.

III. MOTIONS FOR PROTECTIVE ORDER AND TO SEAL

Patent Owner moves for entry of a stipulated protective order and for an order sealing portions of the Patent Owner Response (Paper 21), all of Exhibits 2170–2173, and portions of Exhibit 2080. *See* Paper 22 (attaching stipulated protective order as Appendix A). That motion is unopposed.

A party may move to seal confidential information including, *inter alia*, sensitive commercial information. Consolidated Patent Office Trial

Practice Guide, 19 (Nov. 2019); 37 C.F.R. § 42.54. It is the movant's burden to show good cause for sealing such information, and we balance the party's asserted need for confidentiality with the strong public interest in open proceedings. *Argentum Pharms. LLC v. Alcon Research, Ltd.*, IPR2017-01053, Paper 27 at 4 (PTAB Jan. 19, 2018) (informative).

Patent Owner provides a sufficient explanation for sealing the relevant portions of the Patent Owner Response and the identified exhibits. Exhibits 2170–2173 are license or settlement agreements between Patent Owner and third parties setting forth, for example, payment terms and sales data. Patent Owner states that it produced those agreements with the permission of third parties on the condition that the agreements remain sealed. Paper 22, 3–4. The subject portions of the Patent Owner Response and Exhibit 2080 include discussions about those agreements. Patent Owner has also provided public redacted versions of Exhibit 2080 and the Patent Owner Response (Paper 23) so the record may remain clear and reasonably open.

Patent Owner has established good cause for sealing Exhibits 2170–2173, portions of Ex. 2080, and the Patent Owner Response. The stipulated Protective Order includes minor changes from our default language. That Protective Order, Appendix A to Paper 22, is entered.

Petitioner also filed a Motion to Seal (Paper 31). Petitioner contends that portions of its Reply (Paper 30) and portions of Exhibit 1054 (deposition transcript of Paul K. Meyer) should be sealed because those papers include information that Patent Owner considers contain confidential business information (e.g., licensing practices). Paper 31, 1. Petitioner has

also filed public redacted versions of the Petitioner Reply (Paper 32) and Exhibit 1054. For the same reasons discussed above regarding Patent Owner's Motion to Seal, Petitioner has shown good cause for granting its motion. Petitioner's Reply (Paper 30) and Exhibit 1054 are sealed.

IV. MOTION TO EXCLUDE

Petitioner moves to exclude Exhibit 2301, a deposition transcript of Galla Chandra Rao ("Rao Transcript") taken in connection with the lawsuit between Patent Owner and third party Quest Diagnostics Incorporated. *See supra* Section I(A) (listing related matters). Mot. to Exclude 1–3. Petitioner contends that the Rao Transcript should be excluded as inadmissible hearsay and as irrelevant. Mot. to Exclude 1–3 (citing Fed. R. Evid. 802, 401, 402, and 403).

Because we do not rely on the Rao Transcript in making our determinations in this Final Written Decision, Petitioner's Motion to Exclude is moot and, accordingly, dismissed.

V. CONCLUSION

In Summary:

Claims	35 U.S.C. §	Reference(s)/Basis	Claims Shown Unpatentable	Claims Not shown Unpatentable
81–91, 94–96, 133	103(a)	Chiu, Bianchi		81–91, 94–96, 133
92, 93	103(a)	Chiu, Bianchi, Granger		92, 93
81–96, 133	103(a)	Chiu, Rao		81–96, 133
Overall Outcome				81–96, 133

VI. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the Petitioner has not proved by a preponderance of the evidence that claims 81–96 and 133 are unpatentable;

FURTHER ORDERED that Patent Owner's Motion for Entry of Stipulated Protective Order (Appendix A to Paper 22) and to Seal is *granted*;

FURTHER ORDERED that Petitioner's Motion to Seal (Paper 31) is *granted*;

FURTHER ORDERED that Petitioner's Motion to Exclude (Paper 45) is *dismissed* as moot; and

FURTHER ORDERED that, because this is a Final Written Decision, parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

IPR2021-00902
Patent 7,332,277 B2

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Paper 51
Entered: November 1, 2022

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

LABORATORY CORPORATION OF AMERICA HOLDINGS,
Petitioner,

v.

RAVGEN, INC.,
Patent Owner.

IPR2021-01054
Patent 7,332,277 B2

Before ZHENYU YANG, TIMOTHY G. MAJORS, and DAVID COTTA, *Administrative Patent Judges*.

MAJORS, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining No Challenged Claims Unpatentable
35 U.S.C. § 318(a)
Granting Patent Owner's Motion for Entry of Protective Order and to Seal;
Granting Petitioner's Motion to Seal
37 C.F.R. §§ 42.14, 42.54
Dismissing Petitioner's Motion to Exclude
37 C.F.R. § 42.64(c)

I. INTRODUCTION

Laboratory Corporation of America Holdings ("Petitioner" or "Labcorp"),[1] on June 4, 2021, filed a Petition to institute *inter partes* review of claims 55–63, 66–69, 80, and 127–132 of U.S. Patent No. 7,332,277 B2 (Ex. 1001, "the '277 patent"). Paper 1 ("Pet." or "Petition"). We instituted trial on November 5, 2021. Paper 11 ("Inst. Dec."). During trial, Ravgen, Inc. ("Patent Owner")[2] filed a Patent Owner Response. Paper 18 ("PO Resp."). Later filings include Petitioner's Reply (Paper 29 ("Pet. Reply")) and Patent Owner's Sur-Reply (Paper 37 ("PO Sur-Reply")). An oral hearing was held on July 28, 2022, and a transcript is entered in the record. Paper 50 ("Tr.").

Patent Owner also filed a motion for entry of a protective order and to seal (Paper 19), and Petitioner filed a motion to seal (Paper 30); both motions were unopposed. Petitioner filed a motion to exclude Exhibit 2301 (*see* Paper 42 ("Mot. to Exclude") and Paper 46 ("Mot. Reply")), which Patent Owner opposed (Paper 45 ("Opp.")).

We have jurisdiction under 35 U.S.C. § 6(b). After considering the parties' arguments and evidence, we determine that Petitioner has not proved by a preponderance of the evidence that the challenged claims are unpatentable. *See* 35 U.S.C. § 316(e). Our reasoning is explained below, and we issue this Final Written Decision under 35 U.S.C. § 318(a).

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[1] Petitioner identifies itself as the real party-in-interest. Pet. 1.
[2] Patent Owner identifies itself as the real party-in-interest. Paper 4, 1.

A. Related Patents & Proceedings

The '277 patent issued February 19, 2008, from U.S. Patent Application No. 10/661,165 ("the '165 Application"), filed September 11, 2003. Ex. 1001, codes (21), (22), (45). The '165 Application is a continuation-in-part ("CIP") of Application No. PCT/US03/06198 filed February 28, 2003 ("the '198 PCT" (Ex. 1007)), which claims priority to other applications including U.S. Provisional Application No. 60/378,354, filed May 8, 2002 ("the '354 Provisional" (Ex. 1011)). *Id.* at codes (60), (63); *see also id.* at 1:7–25. The '165 Application is also a CIP of Application No. PCT/US03/27308, filed August 29, 2003 ("the '308 PCT" (Ex. 1009)). *Id.* at 1:14–16. Related U.S. Patent No. 7,727,720 ("the '720 patent") issued on June 1, 2010, and claims priority to some of the same ancestral applications as the '277 patent. Ex. 2041, 1, 4.[3]

The parties identify multiple lawsuits involving the '277 patent. Pet. 1; Paper 4, 1; Paper 14, 1. Those lawsuits include: *Ravgen, Inc. v. Laboratory Corp. of America Holdings*, No. 6:20-cv-00969-ADA (W.D. Tex.); *Ravgen, Inc. v. Quest Diagnostics Inc.*, No. 2:21-cv-09011-RGK-GJS (C.D. Cal.); and *Ravgen, Inc. v. Natera, Inc. and NSTX, Inc.*, No. 1:20-cv-00692-ADA (W.D. Tex.). Paper 4, 1 (listing other lawsuits filed by Patent Owner against, e.g., PerkinElmer, Inc., and Myriad Genetics, Inc.).

[3] For some exhibits herein, we cite the page numbers added to the exhibit copy; we may also use other citation formats (e.g., column and line, paragraph numbers, or original pagination) for some exhibits.

Petitioner and Patent Owner also identify other matters involving the '277 patent pending before the Patent Office. Pet. 2; Paper 4, 1; Paper 10, 1. Petitioner challenges different claims of the '277 patent than challenged here in IPR2021-00902. Claims of the '277 patent have also been challenged in IPR2021-00788, -00789 and -00790 (all filed by Quest),[4] IPR2021-01272 (filed by Illumina, Inc.) and IPR2021-01577 (filed by Streck, Inc.). Pet. 2; Paper 4, 1; Paper 10, 1. We terminated IPR2021-00788 due to settlement prior to reaching a final written decision (IPR2021-00788, Paper 71), a final written decision in IPR2021-00902 is entered concurrent with this decision, and IPR2021-01272 and IPR2021-01577 are ongoing. In addition, Patent Owner identifies *Ex Parte* Reexamination Control No. 90/014,792 as related to the '277 patent. Paper 7, 1. That reexamination is stayed. *See* IPR2021-00902, Paper 24.

The related '720 patent has also been challenged in several matters before the Office: IPR2021-00791 (terminated); IPR2021-01026 (pending); IPR2021-01271 (pending); and *Ex Parte* Reexamination Control Nos. 90/014,703, and 90/014,869 (both stayed).

B. Asserted Grounds of Unpatentability

Petitioner asserts two grounds of unpatentability in this Petition (Pet. 8–9), which are provided in the table below:

Claims Challenged	35 U.S.C. §	Reference(s)/Basis
55–63, 66–69, 80, 127–132	103(a)[5]	Chiu,[6] Lo,[7] Bianchi[8]
55–63, 66–69, 80, 127–132	103(a)	Chiu, Lo, Rao[9]

Petitioner relies on the declarations of Jeremy S. Edwards, Ph.D., among other evidence. Ex. 1002; Ex. 1047. Prior to institution, Patent Owner submitted a declaration from Dr. Glenn D. Prestwich, Ph.D. Ex. 2001. During trial, Patent Owner submitted and relies on a declaration from Dr. Brian Van Ness, Ph.D. (Ex. 2078), among other evidence.[10] The deposition testimony of Drs. Edwards (Exs. 2101, 2299) and Van Ness (Ex. 1045) is also of record.

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[4] On October 19, 2021, we instituted trial in IPR2021-00788, Paper 23 (covering claims 55–63, 66–69, 80–94, 96, and 126–133), and denied institution in IPR2021-00789 (Paper 21) and IPR2021-00790 (Paper 21).
[5] Based on the filing date of the '277 patent, we apply the pre-AIA version of § 103.
[6] Rossa W. K. Chiu et al., *Effects of Blood-Processing Protocols on Fetal and Total DNA Quantification in Maternal Plasma*, 47:9 CLINICAL CHEMISTRY 1607–1613 (2001) (Ex. 1003, "Chiu").
[7] Y. M. Dennis Lo et al., *Quantitative Analysis of Fetal DNA in Maternal Plasma and Serum: Implications for Noninvasive Prenatal Diagnosis*, 62 AM. J. HUM. GENET. 768–775 (1998) (Ex. 1021, "Lo").
[8] Bianchi et al., U.S. 5,648,220, issued July 15, 1997 (Ex. 1004, "Bianchi").
[9] Rao et al., WO 03/018757 A2, published March 6, 2003 (Ex. 1005, "Rao").
[10] The parties submit, for example, additional testimony related to products that are alleged to practice the claimed subject matter and asserted commercial success of such products. *See, e.g.*, Exs. 2080 (Declaration of Paul Meyer) and 2082 (Declaration of Jeffrey Chalmers, Ph.D.).

C. Technology Overview and the '277 Patent

The '277 patent relates to non-invasive methods for sampling DNA and detection of genetic disorders in a fetus. Ex. 1001, 1:31–39. The '277 patent explains that a variety of invasive and non-invasive techniques are available for prenatal diagnosis, including amniocentesis, fetal blood cells in maternal blood, and maternal serum alpha-feto protein. *Id.* at 2:53–57. According to the patent, however, "techniques that are non-invasive are less specific, and the techniques with high specificity and high sensitivity are highly invasive." *Id.* at 2:57–60; *see also id.* at 3:33–37 (citing an increased fetal mortality risk of about 0.5% with amniocentesis).

We provide a brief overview of the components of blood, which is helpful in understanding the challenged claims and the cited prior art. Blood is composed of plasma and other blood components that are suspended in plasma. Ex. 2001 ¶ 35; Ex. 2078 ¶ 22. The major blood components in plasma include red blood cells ("RBCs"), white blood cells ("WBCs") and platelets. *Id.* Although most DNA is typically found inside cells (within the cell membrane and nucleus), some DNA may also be found outside the cells circulating freely in the plasma. *Id.* ¶¶ 22–23. Such circulating DNA is known as "cell-free DNA" ("cfDNA"). *Id.* WBCs, unlike RBCs, include an individual's cellular DNA, and when WBCs are subjected to various stresses (e.g., biological, physical, or chemical), the WBCs may lyse and release additional DNA into the plasma. Ex. 2001 ¶¶ 37–38; *see also* Ex. 1002 ¶ 23 (discussing liberation of DNA from lysis of maternal cells).

By the late 1990s, and prior to the '277 patent, researchers had discovered that pregnant women have cell-free *fetal* DNA ("cffDNA") along

with maternal cfDNA circulating in maternal plasma. Ex. 2001 ¶¶ 36–37; Ex. 2078 ¶ 23. For example, in a study headed by Dr. Dennis Lo, researchers determined that cffDNA was present in maternal plasma in a range of about 3.4%–6.2% (as a percent of total circulating DNA), with the percentages corresponding to early and late pregnancy, respectively. Ex. 1002 ¶ 23 (citing Ex. 1021, 768). At about the same time, researchers determined that, although intact fetal *cells* may also be found in maternal plasma, most fetal DNA in maternal plasma exists in its cell-free form. *Id.*

To analyze cell-free DNA from blood, a blood sample is ordinarily collected (e.g., from a subject's vein), and then further processed. Ex. 2001 ¶ 38. A common mode of preparation for such blood samples involves the use of centrifugation to separate the cellular components and plasma within the sample. Ex. 1002 ¶ 30 (discussing, for example, methods in Chiu); *see generally* Ex. 1003 (describing, *inter alia*, centrifugation and filtration techniques to separate the plasma and cellular fractions).

According to Petitioner's declarant, Dr. Edwards, when working with blood samples, it was known to add blood stabilizing compounds, especially to reduce effects of delayed processing. Ex. 1002 ¶¶ 21–22. Dr. Edwards testifies that known processing "techniques included the use of agents that stabilized the cells and/or analyte(s) and/or prevented coagulation [of the blood] so that samples could be tested, hours, days, or weeks after collection." *Id.* "For example, blood samples were commonly collected in

treated tubes, e.g., EDTA tubes or acid citrate dextrose (ACD) tubes." *Id.* (citing, e.g., Exs. 1003 and 1014).

The '277 patent acknowledges the prior non-invasive use of fetal cells and cell-free fetal DNA, both isolated from maternal blood, for prenatal diagnosis. Ex. 1001, 5:7–59. With regard to fetal cells, the patent notes that the "presence of fetal nucleated cells in maternal blood makes it possible to use these cells for noninvasive prenatal diagnosis," and that such "cells can be sorted and analyzed by a variety of techniques to look for particular DNA sequences." *Id.* at 5:8–13. Yet the patent states that "it is still difficult" to get many fetal cells from maternal blood and "[t]here may not be enough to reliably determine anomalies of the fetal karyotype or assay for other abnormalities." *Id.* at 5:30–34. The patent states that fetal DNA "has been detected and quantitated in maternal plasma and serum" and that "fetal DNA present in the maternal serum and plasma is comparable to the concentration of DNA obtained from fetal cell isolation protocols." *Id.* at 5:39–49. "However," according to the patent, "the diagnostic and clinical applications of circulating fetal DNA is limited to genes that are present in the fetus but not in the mother" and "a need still exists for a non-invasive method that can determine the sequence of fetal DNA and provide definitive diagnosis of chromosomal abnormalities in a fetus." *Id.* at 5:53–59.

The '277 patent describes a method that is said to increase the proportion or percentage of the cffDNA component in a sample from a pregnant female for subsequent analysis. According to the patent, the ability to detect chromosomal abnormalities has been "hindered by the low percentage of free fetal DNA" in maternal samples. Ex. 1001, 89:1–6.

"Increasing the percentage of free fetal DNA would enhance the detection" of trisomy and other genetic abnormalities. *Id.* at 89:6–11.

With the aim of increasing the percentage of cffDNA relative to circulating maternal DNA in a maternal sample, the '277 patent describes adding an agent that inhibits cell lysis. Ex. 1001, 219:38–44 (Example 15) ("[T]he use of cell lysis inhibitors, cell membrane stabilizers, or cross-linking reagents can be used to increase the percentage of fetal DNA in the maternal blood."); *id.* at 89:11–13 ("The percent of fetal DNA in plasma obtained from a pregnant female was determined both in the absence and presence of inhibitors of cell lysis"). The patent explains that, "[w]hile lysis of both maternal and fetal cells is inhibited, the vast majority of cells [in a maternal blood sample] are maternal, and thus by reducing the lysis of maternal cells, there is a relative increase in the percentage of free fetal DNA." *Id.* at 32:36–39. The patent identifies numerous agents as cell lysis inhibitors, cell membrane stabilizers, or cross-linking reagents. *See, e.g.*, *id.* at 31:57–32:21 (listing, for example, formaldehyde, formalin, cleavable crosslinkers, cholesterol, and glucose).

The '277 patent provides results on the use of formalin (i.e., formaldehyde in aqueous solution) as the lysis-inhibiting agent. Ex. 1001, 89:1–91:50 (Example 4). In Example 4, the patent describes collecting a 5 ml blood sample from a pregnant subject, separating the sample into two tubes (each containing EDTA[11]), and adding formaldehyde (25µl/ml) to one

[11] The '277 patent states that EDTA is a "magnesium chelator." Ex. 1001, 31:52–54.

of the tubes. *Id.* at 89:18–25. The samples were centrifuged and 800 µl of each maternal plasma sample was then used for DNA purification and further processing to determine the relative amount of cffDNA present. *Id.* at 89:25–91:13. According to the '277 patent, "the percentage of fetal DNA present in the sample that was treated with only EDTA was 1.56%" and the "percentage of fetal DNA present in the sample treated with formalin and EDTA was 25%." *Id.* at 91:14–20. The percent of total cffDNA in eighteen blood samples with and without formalin was then calculated, with the results (mean percentage cffDNA) provided in Table V. *Id.* at 91:35–43 (reporting, *inter alia*, 19.47% with formalin and 7.71% without formalin), 219:38–226:26 (Example 15).

D. Challenged Claims

Independent claim 55 and several claims that depend (directly or indirectly) from claim 55 are challenged here. Claim 55 reads:

> 55. A method comprising determining the sequence of a locus of interest on free fetal DNA isolated from a sample obtained from a pregnant female, wherein said sample comprises free fetal DNA and an agent that inhibits lysis of cells, if cells are present, wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Ex. 1001, 472:66–473:5.

To illustrate some of the challenged dependent claims, claim 59 depends from claim 55 and adds, "wherein said agent is a cell lysis inhibitor." *Id.* at 473:13–14. Claim 60 depends from claim 59, adding "wherein said cell lysis inhibitor is selected from the group consisting of:

glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, derivatives of formaldehyde, and formalin." *Id.* at 473:15–18.

E. Prosecution History

Challenged claim 55 corresponds to pending claim 58 in prosecution. Ex. 1025, 530.

The Examiner initially rejected pending claim 58 as obvious over "the Lo Patent"[12] combined with other references. *Id.* at 1231. In response, applicant argued that the Examiner had provided no evidence that EDTA in the Lo Patent's samples inhibits cell lysis. *Id.* at 1194–1196, 1199–1200.

The Examiner withdrew the rejections based on the Lo Patent, but entered new rejections for obviousness based on the combination of "Amicucci" or "Umansky," with "Kiessling" (citing Kiessling for its disclosure of formaldehyde as a cell fixative). *Id.* at 927–928, 958–961.

In Remarks dated May 30, 2007, applicant argued that there was no motivation to combine the newly cited references. *See, e.g.*, *id.* at 574–575. Applicant argued that the DNA analyzed in the methods of Umansky and Kiessling was "quite distinct" because Umansky analyzed cell-free fetal DNA circulating outside a cell, "while the DNA analyzed in Kiessling is in and/or is released from a fixed cell." *Id.*; *see also id.* at 593 (advancing similar argument for the Amicucci combination). Applicant also argued that the claimed method addressed a long-felt need and produced unexpected

[12] International Publication No. WO 98/39474 (Ex. 2038). We use the name "the Lo Patent" for Exhibit 2038 to avoid confusing with another reference "Lo" (Exhibit 1021) asserted by Petitioner here.

results. *Id.* at 573–574, 591–592. Specifically, applicant argued that its method was an alternative to invasive prenatal testing, and that, by adding formalin as an agent that inhibits lysis to the maternal sample, the percentage of cffDNA was 25%, compared to 1.56% without formalin. *Id.* at 574 (asserting that, based on prior reports, "the mean percentage of free fetal DNA in a maternal sample was expected to be about 3%").

The Examiner, on September 26, 2007, entered a Notice of Allowability covering claim 58 and the several other claims that ultimately issued. Ex. 1025, 523–525. The Examiner's Reasons for Allowance stated that the various claims are "deemed to be allowable in light of the applicant's amendment filed 30 MAY 07 and the persuasive argument(s) therein." *Id.* at 525.

II. ANALYSIS

A. Principles of Law

"In an [*inter partes* review], the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable." *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify "with particularity . . . the evidence that supports the grounds for the challenge to each claim")).

A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which that

subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) secondary considerations of nonobviousness when presented. *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966). When evaluating a combination of teachings, we must also "determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *KSR*, 550 U.S. at 418 (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)). Whether a combination of elements produces a predictable result also weighs in the ultimate determination of obviousness. *Id.* at 416–417.

B. Person of Ordinary Skill in the Art ("POSA")

In determining the level of skill in the art, we consider the problems encountered in the art, the art's solutions to those problems, the rapidity with which innovations are made, the sophistication of the technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus., Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986).

Petitioner contends a POSA:

> would have had an advanced degree (e.g., M.S. or Ph.D.) in Chemistry, Biochemistry, Molecular Biology, Genetics, Bioengineering, Chemical Engineering, or a related discipline, and at least 2-3 years of experience in a research or clinical laboratory. . . . In addition, a [POSA] would have been familiar with the available techniques for optimizing biological samples to be used in various laboratory analyses, such as for detection of DNA, including cell-free nucleic acids, and would have been

> familiar with the relevant scientific field and its literature at the time when the application was filed, in particular, literature regarding the detection of cell-free nucleic acids.

Pet. 19 (citing Ex. 1002 ¶¶ 12–13). Patent Owner's proposal states that a POSA would have had "a M.D. and/or a Ph.D. in a related area such as genetics, biochemistry, molecular biology, cell biology, or microbiology and at least one to two years of work in one of those related areas." PO Resp. 8. Patent Owner proposes that a POSA could, alternatively, "have a Bachelor's degree in one of the foregoing areas and at least three to four years of work in" such areas. *Id.* (citing Ex. 2078 ¶¶ 16–21).

Patent Owner's proposed POSA level is too broad. Under that proposal, an individual with, for example, an undergraduate degree in microbiology and three years' work experience studying the habitats of bacteria might qualify as a POSA. It is not clear that such person would have sufficient, relevant experience in the detection of chromosomal abnormalities, especially through non-invasive methods for detecting fetal genetic abnormalities in maternal samples, as described in the '277 patent and the prior art here. *See, e.g.*, Ex. 1001, 1:31–5:59 (Field of Invention and Background Art); Ex. 1003 (Chiu). Petitioner's proposal of the POSA level is more precise because it requires familiarity with techniques for detecting cell-free DNA in biological samples, which is relevant to the prior art and the claimed technology. Petitioner's proposal also appears to be more consistent with the cited prior art. *Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001) (explaining that specific findings regarding ordinary skill level are not required where the prior art itself reflects an appropriate

level and a need for testimony is not shown). Accordingly, we adopt Petitioner's POSA level here, but note that our other determinations on this record would not change under Patent Owner's POSA level.[13]

C. Claim Construction

We interpret a claim "using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. 282(b)." 37 C.F.R. § 42.100(b) (2020). Under this standard, we construe the claim "in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." *Id.*

Petitioner does not identify, in the Petition, any terms for which it is seeking express claim construction. Pet. 9. Patent Owner, in its Response, "reserve[d] the right to address claim construction," but similarly did not request interpretation of any claims. PO Resp. 8 n.3.

It is not necessary for us to further construe the challenged claims to resolve this case. Petitioner relies on formaldehyde or formaldehyde donors, as disclosed in Bianchi and Rao, to satisfy the claimed "agent" term, and urges the addition of such compounds to the modified methods of Chiu and Lo for isolating and detecting cffDNA in a maternal sample. There is no dispute on this record that formaldehyde and formaldehyde donors meet the claimed "agent" term. We are, thus, able to determine if the asserted art discloses an "agent" as claimed and whether a POSA would have been

[13] Patent Owner's declarant, Dr. Van Ness, testifies that his opinions would not change under either POSA level. Ex. 2078 ¶ 21.

motivated to add formaldehyde or a formaldehyde donor to Chiu/Lo, which issue we find is decisive here. *See Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co. Ltd.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms that are in controversy, and only to the extent necessary to resolve the controversy.") (internal quotation marks omitted).

D. Overview of the Asserted Prior Art

1. Chiu (Exhibit 1003)

Chiu is an article about molecular diagnostics and genetics, published in Clinical Chemistry in 2001. *See generally* Ex. 1003. Chiu relates to a study on the effects of blood-processing protocols on the quantification of cell-free fetal and total DNA in maternal plasma. *Id.* at 1607–1608 ("[I]t is the objective of this study to investigate the effects of different blood-processing protocols on the quantitative analysis of total and fetal DNA in maternal plasma, as well as the effect on the relative proportions of cellular and cell-free DNA.").

Chiu discloses that "the discovery of fetal DNA in maternal plasma and serum in 1997 . . . [and] numerous reports have confirmed its potential application for noninvasive prenatal diagnosis." *Id.* at 1607. Citing prior studies, Chiu reports that "it has been shown that fetal DNA represents a substantial portion of the total DNA in maternal plasma, contributing ~ 3.4% and ~ 6.2% of total plasma DNA in early and late pregnancy, respectively." *Id.* Based on such prior investigations, Chiu addresses "whether plasma is truly acellular" and "whether fetal DNA circulates predominately in a cellular or cell-free form in maternal plasma." *Id.* at 1608; *see also id.* at

1607 (disclosing, as background, that "[r]ecently, apoptotic cells have been found in plasma obtained by centrifugation of blood from pregnant women, raising the question of what constitutes plasma and whether plasma is truly cell free"). Because studies may rely on quantification of fetal DNA in maternal plasma, Chiu discloses that "it would be of prime importance to investigate whether the apparent maternal plasma DNA concentration would be affected by different sample processing protocols." *Id.* at 1608.

Chiu discloses the use of different protocols to process blood samples and separate maternal plasma; those protocols include centrifugation, microcentrifugation, and filtration. *Id.* at 1608–09. Certain genes (β-globin and *SRY*)[14] in the separated plasma were then isolated and amplified via PCR for determination of the concentrations (genome-equivalents/mL) of those genes in the samples. *Id.*

From the data, Chiu discloses that "different blood-processing protocols have a significant impact on the quantification of *β-globin*, but not *SRY* sequences in plasma." *Id.* at 1612. "In other words, by altering the blood-processing protocol, quantification of total, but not fetal, DNA is affected." *Id.* Chiu explains, for example, that "centrifugation alone, by various speeds (1600*g* and 800*g*) led to total DNA concentrations that were significantly different and higher than those of filtered plasma ($P < 0.05$)." *Id.* Chiu teaches, "[t]herefore, it can be deduced that despite centrifugation,

[14] These genes could be used as proxies in Chiu's methods for determining the amount of fetal to total DNA because the β-globin gene is present in maternal and fetal DNA, and the *SRY* gene only in the fetal DNA. Ex. 1003, 1608, 1612.

some of the maternal cells could remain in plasma, leading to an increase in the total DNA in plasma." *Id.* ("[C]entrifugation alone is not effective in removing all of the cells in plasma, and the number of cells that remain in plasma after processing is variable."). Chiu teaches that the "lack of difference in fetal DNA concentration among the different [sample-processing] treatment groups . . . suggests that most of the fetal DNA circulates in an extracellular form." *Id.* ("[I]ntact fetal cells contribute only a very small proportion of the quantifiable fetal DNA"); *see also id.* (disclosing that "fetal cells are detectable at a frequency of . . . ~ 2 fetal cells/mL of Percoll-derived maternal plasma"). Chiu concludes that "[d]ifferent protocols of blood sample processing impart a significant effect on the quantification of total DNA in maternal plasma." *Id.* at 1613.

Although Chiu indicates that an initial centrifugation may not, alone, be sufficient to remove cells from the plasma samples, Chiu teaches that cell-free samples may be obtained with additional physical processing steps—filtration or microcentrifugation. *Id.* at 1609–13. More specifically, Chiu discloses that "[p]lasma filtration by a submicron filter is used to remove residual cells that remain in plasma after the initial centrifugation step" and that "the DNA concentration in filtered plasma reflects the proportion of 'extracellular' fetal and total DNA in the blood sample." *Id.* at 1612. Chiu teaches that "[b]ecause plasma subjected to microcentrifugation [(i.e., centrifugation at 16,000*g*)] . . . consistently leads to a total DNA concentration that is statistically similar to that of filtered plasma, we infer that microcentrifugation is just as effective at generating cell-free plasma as filtration." *Id.*; *see also id.* at 1613 ("Virtually cell-free plasma can be

obtained by centrifugation of blood samples, followed by filtration or microcentrifugation.").

Chiu closes its discussion with the following disclosure:

> By highlighting the importance of centrifugation protocols for plasma processing, our data have obvious bearing on this type of analysis [(analysis of free circulating nucleic acids, including, e.g., fetal DNA, and plasma DNA used for cancer diagnosis)]. As research in the field of circulating nucleic acids is growing rapidly for findings to be easily comparable across studies, some form of standardization needs to be agreed on.

Id.

2. Bianchi (Exhibit 1004)

Bianchi is a U.S. patent that issued in 1997. Ex. 1004, code (45). Bianchi discloses methods for labeling intracytoplasmic target molecules, e.g., a protein or a nucleic acid, in order to determine whether cells having such a target molecule are present in a sample. *Id.* at Abstr., 2:14–39 (listing target molecules, such as fetal hemoglobin that is characteristic of fetal cells (e.g., fetal nucleated erythrocytes) in a maternal blood sample). Bianchi describes the applicability of its methods to prenatal diagnosis ("allow[ing] single-cell genetic and chromosomal analysis which can be used for, e.g., prenatal diagnosis"). *Id*. at 2:66-3:1.

Bianchi teaches that there are two key features of its method. First, that the intracytoplasmic target molecule can be labeled within the cell. *Id.* at 3:24–32 ("First, the plasma membrane is sufficiently permeable so that a reagent capable of detectably labeling the target molecule is able to traverse the plasma membrane into the cytoplasm."). Second, that "the plasma

membrane is sufficiently intact so that substantially all of the intracytoplasmic target molecule and the DNA of the target cell remain in the cell." *Id.* at 3:32–35.

Bianchi discloses use of a reagent system and sample treatment process for its "permeabilization method" where the cell preparation sample is incubated in 2–8% (w/v) paraformaldehyde for between 10 minutes to 4 hours at, preferably, about 37° C. *Id.* at 3:37–42. The cell preparation suspected of including the target cell is "then is incubated permeabilized" in a solution containing alcohol (for example, incubating the sample in methanol:acetone at about 4° C). *Id.* at 3:44–53 (following permeabilization, the cells are washed and contacted with a labelling reagent, such as an antibody, which can be used for detecting the target cell from the cell preparation).

3. Rao (Exhibit 1005)

Rao is an international patent application that published on March 6, 2003. Ex. 1005, code (43).[15] Rao relates to "[c]ompositions and methods for stabilizing rare cells in blood specimens," and compositions that "serv[e] as cell fixatives" to "minimize losses of target cells (for example, circulating tumor cells [(CTCs)]) and formation of debris and aggregates from target cells." *Id.* at Abstr. (teaching that the cells are stabilized so the rare cells can be better detected or enumerated).

[15] Rao includes a priority claim to two U.S. provisional applications (Application No. 60/314,151, filed August 23, 2001 (Ex. 1048), and Application No. 60/369,628, filed April 3, 2002).

Rao discloses that tumor cells undergoing apoptosis have altered membrane permeability that may lead to the escape of DNA and other cellular components. *Id.* at 2:5–10 ("Such tumor cell debris may still bear epitopes that are characteristic of intact cells, and can lead to spurious increases in circulating cancer cells."). Rao teaches that "[l]eukocytes . . . are known to be labile and diminish on storage," thus "increas[ing] the amount of cellular debris, derived from normal blood cells or proteins were found to interfere with the isolation and detection of rare target cells such as CTC." *Id.* at 2:14–17.

Rao discloses that "[t]here is a large body of published or patented art regarding the stability and stabilization of normal blood cells over time and several proprietary commercial stabilizers are available for preserving white blood cells." *Id.* at 3:4–8 (listing stabilizers, including "Cyto-Chex™ from Streck Laboratories"). Rao discloses that, "[d]espite the shortcomings of paraformaldehyde or reagents containing paraformaldehyde, formaldehyde, glutaraldehyde and glyoxal, such reagents are frequently used for fixing and stabilizing tumor cells in blood or histology specimens." *Id.* at 3:16–18.

Rao discloses that the "ideal" stabilizer preserves the target cells while minimizing interfering cellular debris in a sample. *Id.* at 7:20–24. Rao identifies "the formaldehyde donor imidazolidinyl urea" as being "effective" at a preferred concentration of 0.1–10% by volume of the specimen. *Id.* at 8:2–5, 22:1–14 (claim 1 composition, including a stabilizing agent), claim 6 (said stabilizing agent "is a formaldehyde donor")).

4. Lo (Exhibit 1021)

Lo is an article published in 1998 in The American Journal of Human Genetics. Ex. 1021. Lo discloses a "real-time quantitative PCR assay to measure the concentration of fetal DNA in maternal plasma and serum." *Id.* at 768. According to Lo, analysis of fetal DNA can be used for "fetal sex determination" and for "prenatal diagnosis" of various disorders, including "sex-linked disorders," "autosomal dominant disorders," and "autosomal recessive genetic disorders" like "certain hemoglobinopathies," and "cystic fibrosis." *Id.* at 773–774. In Lo's study, peripheral maternal blood samples were taken from pregnant women. *Id.* at 769 (Subjects and Methods). Lo teaches that fetal DNA can be detected in both maternal plasma and maternal serum. *Id.* at 772 ("The high concentration of fetal DNA in maternal plasma and serum has allowed us to detect reliably the presence of fetal genetic material."). However, maternal serum has a higher quantity of "background maternal DNA" compared to plasma. *Id.* Therefore, according to Lo, it may be preferable to use maternal plasma rather than maternal serum for "robust fetal DNA detection." *Id.*; *see also* Ex. 1002 ¶¶ 32–34 (analyzing Lo and opining "Lo suggests that plasma may be preferable to serum for robust fetal DNA detection because of the higher occurrence of lysed cells in serum").

E. Obviousness over Chiu, Lo, and Bianchi

Petitioner asserts that claims 55–63, 66–69, 80, and 127–132 are unpatentable as obvious over the combination of Chiu, Lo, and Bianchi.

Pet. 20–41; *see id.* at 21–28 (independent claim 55), 29–41 (claims depending from claim 55).

As we discuss in our analysis below, this ground turns on whether Petitioner has met its burden to establish, by a preponderance of the evidence, that the POSA would have found it obvious to combine and modify the asserted art. More specifically, the ground turns on whether it would have been obvious to modify the maternal blood processing and cffDNA detection method of Chiu and Lo to include paraformaldehyde as a fixative agent, as allegedly disclosed in Bianchi, in the manner proposed by Petitioner.

We gave an overview of the asserted prior art above. Below, we review Petitioner's contentions on claim 55 and Patent Owner's counterarguments. We will then turn to our analysis.

1. Petitioner's Contentions on Claim 55

a) *"A method comprising determining the sequence of a locus of interest on free fetal DNA isolated from a sample obtained from a pregnant female"*

Petitioner contends that the combination of Chiu and Lo teach a method that includes the "determining" step of claim 55. According to Petitioner, Chiu and Lo both describe isolating DNA from a pregnant female's blood sample, and detecting fetal nucleic acids (free and cellular) in the sample by subjecting the isolated DNA to real-time PCR. Pet. 22–24 (citing, e.g., Ex. 1003, 1607–1608; Ex. 1021, 769; Ex. 1002 ¶¶ 29–31, 38–41). Petitioner cites Chiu's teachings related to the processing of a maternal blood sample to produce plasma, such as by means of centrifugation and

microcentrifugation, or centrifugation and filtration, and the effects of such processing methods on total and fetal DNA in the plasma sample. *Id.* at 22, 25–26 (citing, e.g., Ex. 1003, 1607–1608; Ex. 1002 ¶¶ 29–31). Petitioner alleges that "Lo further suggests detecting sequences at a particular locus of interest on a gene by detecting polymorphisms and mutations within a gene, including those associated with sex-linked disorders and hemoglobinopathies." *Id.* at 24 (citing Ex. 1021, 773–774; Ex. 1002 ¶ 38).

b) "wherein said sample comprises free fetal DNA and an agent that inhibits lysis of cells, if cells are present, wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor"

For the two "wherein" clauses of claim 55, Petitioner contends that "Chiu and Lo are silent regarding whether the sample comprising free fetal DNA contains an agent, besides EDTA, that inhibits cell lysis, wherein the agent is a membrane stabilizer, cross-linker, or cell lysis inhibitor." Pet. 25 (citing Ex. 1002 ¶ 40).[16] According to Petitioner, "Chiu discloses the blood samples should be processed to obtain plasma within two hours of collection, suggesting that EDTA tubes may not be sufficient for processing after two hours," and Chiu also "notes that apoptotic cells may be present in

[16] Chiu and Lo include EDTA as an anticoagulant to prepare plasma. Ex. 1003, 1608; Ex. 1021, 769 (disclosing that maternal blood was collected in EDTA tubes and then centrifuged to separate plasma from the cellular component). Petitioner has not asserted in this case that EDTA is encompassed by the "agent" limitation of claim 55.

plasma from blood of pregnant women when the samples are processed only by [an initial] centrifugation." *Id.* at 25–26 (citing Ex. 1002 ¶ 40).

Petitioner turns to Bianchi for its alleged teaching of an "agent that inhibits lysis of cells" as recited in claim 55. According to Petitioner, Bianchi teaches a method where rare cells are permeabilized so target molecules within such cells can be labeled, and cells containing such targets can be identified in a sample. *Id.* at 26; *see* Ex. 1004, 2:14–30 (identifying intracytoplasmic molecules like proteins, and disclosing "fetal hemoglobin" as a preferred target). Petitioner cites Bianchi's teachings on permeabilizing the membrane of cells suspected of containing the target molecule by incubating the cells in a stepwise preparation of paraformaldehyde and alcohol at certain concentrations, times, and temperatures. Pet. 26 (citing Ex. 1004, 3:25–43; Ex. 1002 ¶¶ 35, 41); *see also* Ex. 1004, 3:48–53 (disclosing that, after permeabilizing the cells, a labeling reagent (such as an antibody) is introduced to label the target molecule). Petitioner asserts that "[p]araformaldehyde is a polymer of formaldehyde" and cites the '277 patent's identification of formaldehyde and its derivatives as being cell lysis inhibitors. Pet. 26 (citing Ex. 1027; Ex. 1002 ¶ 35; Ex. 1001, 6:55–60). Petitioner further notes two features essential to Bianchi's method: the membrane of the cells must be made sufficiently permeable so that reagents that detectably label the target molecule can traverse the membrane and enter the cytoplasm; and the membrane must remain sufficiently intact so that substantially all the intracytoplasmic molecule and DNA remain within the target cell. *Id.* at 26 (citing Ex. 1004, 3:25–35); *see also* Ex. 1004, 1:35–

49 (defining "substantially all of the intracytoplasmic target molecule and the DNA of the cell").

c) Motivation to Combine and Reasonable Expectation of Success

At a high level, Petitioner argues that a POSA would have been motivated to modify the method of Chiu and Lo to include paraformaldehyde,[17] as disclosed in Bianchi. Pet. 26–28. The details of Petitioner's motivation-to-combine theory are discussed below.

Petitioner first contends that a POSA would have been motivated to combine Chiu and Lo because they "report similar methods and include an overlapping set of authors." *Id.* at 27. And, Petitioner asserts, a POSA would have been motivated to combine Chiu and Lo "to determine the sequence of a locus of interest on free fetal DNA isolated from a sample obtained from a pregnant female." *Id.* (citing Ex. 1002 ¶ 38 (testifying that "[b]oth methods detect the *SRY* gene (specific for a male fetus) and the *β-globin* gene (representative of total DNA) using a sequence detector.")).

Petitioner contends that it was known "that fetal DNA exists mainly in cell-free form and is not released significantly from dead or dying cells in the maternal circulation." Pet. 27. Petitioner contends that it was also known that "the amount of free maternal DNA was significantly increased [in samples] by liberation of DNA from maternal cells lysed during clotting." *Id.*; Ex. 1003, 1607; Ex. 1002 ¶¶ 40–41. In support of Petitioner's

[17] Petitioner has described paraformaldehyde as both a "polymerized form" of formaldehyde and as a "formaldehyde donor." *See* Pet. 26, 45–46.

arguments, Dr. Edwards opines that, when carrying out a method of processing maternal plasma for analysis of cffDNA (like Chiu), a POSA "would have been motivated to improve the detection of free fetal nucleic acids, for example, by increasing the percentage of fetal nucleic acids with respect to the total nucleic acids present by minimizing the introduction of any cellular DNA into the plasma sample." Ex. 1002 ¶ 23; *see also id.* ¶ 40 (opining that a POSA "would have recognized a need to reduce cell lysis, as cell lysis was known to affect measurements of extracellular components").

Petitioner contends that "Chiu discloses centrifugation and/or filtration as a means for reducing" maternal cells and maternal DNA in the assay background. Pet. 27–28 (citing Ex. 1002 ¶ 40). According to Petitioner, "Chiu also suggests that . . . processing in EDTA tubes may not be sufficient for processing after two hours." *Id.* Petitioner contends that a POSA would have been motivated "to try other means for reducing background, such as the inclusion of agents to inhibit the lysis of the maternal cells." *Id.* at 28. Petitioner argues a POSA would have sought to determine "whether use of such agents" work "as an alternative to or in combination with EDTA, because it would eliminate or reduce apoptosis of maternal and fetal cells during sample storage or processing (thereby eliminating or reducing background) and would reduce the steps of preparing the sample for analysis (i.e., by eliminating a second centrifugation or filtration step)." Pet. 28 (citing Ex. 1002 ¶¶ 41–42); *see*

also id. at 26 (arguing "Bianchi provides such an alternative" with paraformaldehyde in an alleged "stabilization step").

Petitioner further argues that a POSA "would have had a reasonable expectation that use of paraformaldehyde in place of the centrifugation and/or filtration steps in Chiu's methods would have been successful for isolating and detecting fetal nucleic acids . . . as both techniques would have been expected to reduce background noise resulting from lysis of cells in the sample." Pet. 28; Ex. 1002 ¶ 41.

Petitioner later expounds on its theory about the modification of Chiu's and Lo's methods in view of Bianchi and why the POSA would allegedly have been motivated to make those changes.[18] According to Petitioner, a POSA would, based on "[g]eneral knowledge," have known "that blood samples, particularly white blood cells, were stable for no more than twenty-four to forty-eight hours." Pet. Reply 17 (citing Ex. 1049,[19] 2:4–12). Moreover, Petitioner asserts, samples that required highly technical

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[18] Petitioner asserts in Reply that Lo's disclosure that cffDNA must be present "in sufficient quantities for reliable molecular diagnosis to be carried out" (Ex. 1021, 768) suggests that other enrichment may be needed, and that "[t]his problem is distinguishable from high background" due to lysis. Pet. Reply 17. Chiu and Lo both include a secondary centrifugation step, and Dr. Edwards opines that "[t]hese steps undoubtedly result in some residual fetal DNA left behind in each step" and "[g]iven the limited amount of fetal DNA, a POSITA would have been motivated to avoid the second centrifugation and removal step." Ex. 1047 ¶ 16.

[19] Exhibit 1049 is U.S. Patent No. 5,849,517, which issued December 15, 1998, and is assigned to Streck Laboratories, Inc. Ex. 1049, codes (45), (73). Exhibit 1049 was not cited or submitted with the Petition.

genetic analyses were "routinely shipped to . . . larger laboratories." *Id.* at 18. Thus, Petitioner contends, a POSA "would have looked to known methods for stabilizing blood samples to enable shipment of samples to central laboratories." *Id.* (citing Ex. 1002 ¶¶ 21–22); *see also id.* at 17–19 (citing Ex. 1047 ¶¶ 15–17[20]). Allegedly, a POSA, "starting from Chiu in combination with Lo would have looked to references, such as Bianchi . . . regarding stabilization of cells and general knowledge in the art regarding instability of blood samples." *Id.* at 18–19 ("[U]sing general knowledge in

[20] Dr. Edwards's testimony similarly refers to a need to ship samples to centralized labs because of the "relatively low total amount of cell free fetal DNA and its relatively low percentage of total DNA in the blood and given the complexity of the genetic analysis." Ex. 1047 ¶ 15. Dr. Edwards further opines that "centrifuges and filtration are not available at all blood collection sites and . . . that such steps could decrease the amount of the sample and, thus, the amount of available fetal DNA" such that a POSA "would have been motivated to eliminate any steps that reduce the amount of fetal DNA." *Id.* Extending this underlying testimony suggests that a POSA would modify the methods of Chiu/Lo to omit on-site physical steps like microcentrifugation or filtration because, according to Dr. Edwards, those steps "could decrease the amount of the sample and, thus, the amount of available fetal DNA." *Id.* Dr. Edwards provides no data or persuasive independent factual evidence, however, to support his opinion that *removing* the centrifugation or filtration steps of Chiu/Lo, which steps produced "cell-free" plasma samples with improved proportional recovery of detectable cffDNA (*see* Ex. 1003, 1609, 1613), would have been understood as providing means for greater total or proportional recovery of detectable cffDNA. Ex. 1047 ¶¶ 15–16; 37 C.F.R. § 42.65(a) (instructing that expert testimony that does not disclose underlying facts or data in support "is entitled to little or no weight").

the art, [a POSA] would have selected the fixation step of Bianchi . . . to stabilize the cells.").[21]

Although Petitioner and its declarant, Dr. Edwards, acknowledged during trial that formaldehyde can potentially damage DNA, Petitioner nevertheless asserts that "the use of formaldehyde as a nucleic acid fixative was fully characterized." Pet. Reply 19–20; Ex. 2299, 57:23–58:4 (testimony of Dr. Edwards agreeing that formaldehyde can damage DNA including cell-free DNA). Petitioner cites the Srinivasan[22] review article for its disclosure of "criteria recommended in the literature for use of formaldehyde as a tissue nucleic acid fixative." Pet. 20 (quoting Ex. 1051, 1965–1966) (disclosing, among other criteria, a "minimal prefixation time lag, < 2 hours" and total "duration of fixation (3 to 6 hours)"). Thus, Petitioner argues a POSA "would have been motivated to try and would

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[21] Patent Owner criticizes Petitioner's use of "newly-cited evidence and vague reliance on 'general knowledge' and 'creativity'" in the Reply as an improper and untimely attempt by Petitioner to fill holes in its theory. *See, e.g.*, PO Sur-Reply 3–5 ("Petitioner adds a new theory to support modifying Chiu/Lo based on 'general knowledge' and an alleged known need for 'shipment of samples to central laboratories.'"). We also have concerns about the evolution of Petitioner's obviousness challenge during this proceeding and about the timeliness of the submission of certain evidence in support of it. Petitioner's obviousness challenge is, in any event, unpersuasive for reasons we discuss below in our analysis.

[22] Mythily Srinivasan et al., *Review: Effect of Fixatives and Tissue Processing on the Content and Integrity of Nucleic Acids*, 161:6 American Journal of Pathology, 1961–1971 (Dec. 2002) (Ex. 1051 or "Srinivasan"). Petitioner filed Srinivasan with its Reply. Srinivasan is also of record as Exhibit 2150, which Patent Owner filed with its Response.

have had a reasonable expectation of success in using formaldehyde or a formaldehyde donor to stabilize white blood cells in a maternal blood sample,"[23] and a POSA "would have understood that, although long durations or high concentrations of formaldehyde should be avoided, samples promptly processed and fixed yielded reliable nucleic acid analysis." *Id.* at 19–20 (citing Ex. 1047 ¶ 18).

At the oral hearing, Petitioner further clarified its motivation-to-combine story. Tr. 17:4–19:13. According to Petitioner, Chiu's approach addressed possible maternal cell lysis with a sophisticated "research laboratory method"—involving "rapid processing" and either filtration or microcentrifugation after an initial centrifugation to produce essentially cell-free plasma. *Id.* at 17:4–19:13. But, Petitioner contends, Chiu's approach did not address "a real-world problem" where blood is "collected at remote locations" and would, thus, need to be shipped to a central laboratory for processing and cell-free DNA analysis. *Id.* So, under its theory, Petitioner "envision[s] . . . that there would be a need to eliminate this immediate processing [of Chiu]," the blood-collection "tube could either contain the formaldehyde or formaldehyde donor . . . or it could be added to the blood after collection," and "[e]ither way, the point would be to then be able to transport it where, at a central location, it could be further processed because, naturally, it's going to have to be centrifuged to -- to achieve plasma." *Id.*

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[23] As Petitioner's counsel confirmed, its theory is based on addition of formaldehyde or formaldehyde donors, not other agents. Tr. 22:24–23:17.

For the reasons above, Petitioner contends that claim 55 would have been obvious over Chiu, Lo, and Bianchi.

2. Patent Owner's Arguments

Patent Owner raises several arguments why a POSA would not have been motivated to combine and modify the asserted prior art in the manner proposed by Petitioner with a reasonable expectation of success. PO Resp. 8–25; PO Sur-Reply 2–13. We focus our discussion on two of Patent Owner's arguments, discussed in detail below, because we find such argument and related evidence decisive on this record.

First, Patent Owner argues that a POSA would not have combined Bianchi with Chiu and Lo because doing so would have been thought to permeabilize (add gaps or holes in) maternal cells in the blood sample of Chiu/Lo, allowing DNA to escape and diluting the extracellular fraction of the sample, contrary to Chiu's goals. PO Resp. 15–20.

According to Patent Owner, Bianchi's cell-focused, cell-isolation protocols are fundamentally different from Chiu's and Lo's methods for analyzing cell-free DNA in a maternal plasma sample. *Id.* at 15; PO Sur-Reply 7–8 (citing Ex. 2299, 152:21–155:3) (Dr. Edwards's testimony that Bianchi's paraformaldehyde is added to isolated mononuclear cells resuspended in a buffer—not blood samples that contain free nucleic acids)). Patent Owner contends that Bianchi is unconcerned with analysis of any extracellular blood component, much less cell-free DNA or cffDNA; as Patent Owner notes, the extracellular fraction in Bianchi is washed away and "simply discarded." PO Resp. 15–16 (citing Ex. 1004, 6:1–14); Ex. 1025, 574, 593; Ex. 2078 ¶ 101). Also, Patent Owner contends, Bianchi is not

concerned with reducing extracellular background from released DNA as evidenced by the fact that Bianchi's method "***permeabilizes cells***" so that materials can traverse the cell membrane. *Id.* at 16.

Patent Owner contends Bianchi's permeabilization approach runs contrary to the goal of reducing DNA background in the extracellular fraction of the sample, as in Chiu's or Lo's methods for analyzing cffDNA. PO Resp. 17–19. Although Bianchi endeavors to retain "substantially all" the target analytes as well as the DNA within the target cells, Patent Owner contends that "even in the best scenario, its methods allow cellular contents—including DNA—to escape." *Id.* at 17–18 (citing Ex. 1004, 1:42–48, 3:32–35; Ex. 2078 ¶¶ 104–108). Patent Owner cites Bianchi's disclosure that as much as 50% of the cellular molecules and DNA can escape notwithstanding Bianchi's preference for lower percentages down to about 1%. *Id.* (citing Ex. 1004, 1:42–48). Citing Dr. Van Ness's testimony, explaining the improbability that such lower DNA leakage rates would be expected in practice, Patent Owner contends that "[e]ven if the most preferred percentages were achieved, . . . a POSA would have appreciated that releasing 1% of cellular DNA in a sample" would add background DNA and "significantly dilute[] the cell-free fetal DNA Chiu and Lo seek to detect." *Id.* at 17–19 (citing Ex. 2078 ¶¶ 103–110 (Dr. Van Ness's testimony explaining why this would frustrate Chiu's goals and method, greatly diminishing cffDNA concentrations); Ex. 2101, 107:21–108:17, 113:23–114:5 (Dr. Edwards's testimony that "any" leakage will "cause the fetal fraction to go down")).

Patent Owner also contends that Petitioner's theory "cherry-picks" paraformaldehyde from Bianchi and mischaracterizes the paraformaldehyde incubation as a "stabilization step." PO Resp. 19–20. According to Patent Owner, "Bianchi incubates with paraformaldehyde as part of the '***permeabilization*** method' (Ex. 1004, 3:36–43, Claim 8) and never describes paraformaldehyde as 'stabilizing' cells." *Id.* ("Bianchi provides no information about how that paraformaldehyde solution would affect cells without the permeabilizing reagents in Chiu and Lo's methods."). In any case, Patent Owner contends, even if a POSA would have understood that paraformaldehyde performs some cell-stabilizing function, Petitioner's own expert admitted on cross-examination that Bianchi's paraformaldehyde treatment "will cause gaps in the [cell] membrane." *Id.* (quoting Ex. 2101, 102:15–103:20); *see also* Ex. 2101, 38:11–39:20 (testimony of Dr. Edwards that "there will probably be holes in the membrane when you add [formaldehyde] to a cell.").

Second, Patent Owner argues that a POSA would not have been motivated to use Bianchi's formaldehyde-based reagent in a modified Chiu/Lo method, and would not have reasonably expected success in doing so, because it was known that formaldehyde can damage DNA, and the cffDNA of Chiu/Lo is already a very scarce analyte. PO Resp. 21–25. As Patent Owner notes, "reasons to combine cannot be viewed in a vacuum apart from evidence suggesting reasons not to combine." *Id.* (quoting *Arctic*

Cat Inc. v. Bombardier Recreational Prods. Inc., 876 F.3d 1350, 1363 (Fed. Cir. 2017)).

According to Patent Owner, many sources reported on formaldehyde's detrimental effects on nucleic acids, undermining Petitioner's reasons for adding it to the methods of Chiu/Lo. *Id.*; Ex. 2078 ¶¶ 113–117. Patent Owner and Dr. Van Ness explain that interactions between formaldehyde and cell-free DNA, let alone cffDNA in plasma, were previously unknown (Ex. 2078 ¶¶ 115–116); but even as to DNA more generally, studies warned against using formaldehyde due to potential harm to nucleic acids. PO Resp. 22. The Srinivasan review article, for instance, states that "[a] method to overcome the problems of formaldehyde is to use an alternative fixative that is better suited for the preservation of nucleic acids and proteins." Ex. 2150, 1966; *see also id.* at 1964–65 (noting "considerable evidence suggests that formaldehyde induces DNA degradation" and "formaldehyde initiates DNA denaturation . . . at the AT-rich regions of double-stranded DNA creating sites for chemical interaction").

According to Patent Owner, interactions between formaldehyde and DNA were known to arise from a number of detrimental effects. PO Resp. 22–23; *see, e.g.*, Ex. 2139, 945 ("Swenberg"). Swenberg, for example, discloses "single-stranded DNA breaks," "DNA-protein crosslinks," "sister chromatid exchanges," and "chromosome aberrations and mutations"

resulting from the use of formaldehyde as a cellular fixative.[24] Such concerns about the potential for DNA damage, in the opinion of Dr. Van Ness, would have dissuaded the POSA from using formaldehyde (or paraformaldehyde) for a novel application with cffDNA detection and analysis. PO Resp. 23–24 (citing Ex. 2078 ¶¶ 115–116). As Patent Owner explains, the analysis of cffDNA was a completely new and developing field. *Id.* (explaining that, before the late 1990s and discovery of cffDNA circulating in plasma, the plasma portion of the sample was "***routinely discarded***" (quoting Ex. 2038, 2:5–9)).

Continuing, Patent Owner argues, Petitioner has produced no evidence suggesting a use of formaldehyde in the context relevant here—for the preparation and analysis of cell-free nucleic acids. PO Sur-Reply 10–11. Patent Owner notes the many differences between cellular DNA and cell-free DNA, including that the latter is smaller, limited in quantity, and easily damaged. *Id.* at 12–13 (citing Ex. 2086 ¶ 4; Ex. 2299, 28:19–30:22

[24] Patent Owner cites several other references as evidencing the problems with formaldehyde's use, including damage to nucleic acids. Although such references do appear to report consistently on drawbacks with formaldehyde, many of those references post-date the filing date of the '277 patent by years. *See, e.g.*, PO Resp. 23–24 (citing Ex. 2155, published in 2005); Ex. 2047, published in 2018. For purposes of this decision, we do not rely on these references.

(testimony of Dr. Edwards that "the list is quite long in terms of the differences")).[25]

Patent Owner contends that Petitioner's theory that a POSA would have expected that formaldehyde could be used safely and effectively with cell-free fetal DNA analysis does not hold up to scrutiny. PO Sur-Reply 10–13. Patent Owner cites Dr. Edwards's admission that formaldehyde can damage DNA, including cell-free DNA. *Id.* at 10–11 (citing Ex. 2299, 57:23–58:4). Patent Owner argues that Petitioner's citation to Srinivasan does not fill the holes in Petitioner's theory because Srinivasan does not discuss cell-free DNA at all; if anything, Srinivasan "urges avoiding" formaldehyde even for "***tissue*** [(i.e., cellular)] ***nucleic acids***." *Id.* (citing Ex. 1051, 1964–1966; Ex. 2299, 41:17–25). On whether a POSA would be unconcerned with formaldehyde's problems or just "tailor" conditions (time of fixation, concentrations, etc.) to address them (*see, e.g.*, Pet. Reply 20), Patent Owner contends that notion is at odds with testimony of Petitioner's own expert in related litigation. *Id.* at 9–10 (citing Ex. 2300 ¶ 390

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[25] Patent Owner also points to a recent Board decision as recognizing the differences between cellular and cell-free DNA, and whether fixatives are interchangeable for use with them. PO Sur-Reply 12–13 (citing *Ex Parte Fernando*, Appeal No. 2021-003268, 2022 WL 855866, at *12 (PTAB Mar. 21, 2022) (crediting the argument that "stabilization methods suitable for stabilization of blood samples do[] not necessarily translate into suitable stabilization methods for cell-free fetal nucleic acids")). That case relates to an appeal of the Office's rejection of claims in a January 19, 2010, patent application owned by third-party Streck, Inc. Ex. 2298, 1–2. That decision was based on specific arguments raised in that case and the record developed there; it does not control the outcome in this IPR.

(testimony of Dr. Larry Dumont about formaldehyde degrading nucleic acids and inhibiting PCR, even at low concentrations)). Moreover, Patent Owner contends, Petitioner's invocation of Srinivasan's "criteria" for using formaldehyde (e.g., limiting exposure to "3 to 6 hours" to reduce DNA damage) conflicts with Petitioner's proffered motivation to stabilize blood samples for longer than 24–48 hours, so that samples may be shipped away to a central lab for centrifugation and cffDNA analysis. *Id.* at 11–12. According to Patent Owner, Petitioner never clarifies how the POSA would apply such "criteria" to stabilize blood for the long durations of formaldehyde exposure contemplated by Petitioner's modified Chiu/Lo/Bianchi method. *Id.*

For at least the above reasons, Patent Owner contends Petitioner has not met its burden to prove, by a preponderance of the evidence, that claim 55 is unpatentable as obvious over Chiu in view of Lo and Bianchi.

3. Analysis

On this record, we agree with Patent Owner that Petitioner has not carried its burden to establish that a POSA would have been motivated to add paraformaldehyde to the modified blood sampling and cffDNA detection method of Chiu/Lo.[26] More specifically, we find on this record

[26] *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1327 (Fed. Cir. 2016) ("Whether an ordinarily skilled artisan would have been motivated to modify the teachings of a reference is a question of fact.") (citations omitted); *Alza Corp. v. Mylan Labs., Inc.*, 464 F.3d 1286, 1289 (Fed. Cir. 2006) ("The presence or absence of a motivation to combine references in an obviousness determination is a pure question of fact").

that a POSA would have been dissuaded from adding Bianchi's paraformaldehyde because the POSA would have expected Bianchi's paraformaldehyde to create gaps in the cell membranes, providing a means for maternal DNA to escape into the sample. We also find on this record that a POSA would have been dissuaded from adding paraformaldehyde to the modified method of Chiu/Lo because formaldehyde was known to damage nucleic acids.[27] Patent Owner's reasoning and evidence on those issues, separately and cumulatively, outweigh Petitioner's comparatively weak showing on whether a POSA would have combined the art in the manner proposed. We discuss in greater detail below.

We begin with the issue that paraformaldehyde contributes to permeabilization of the cellular membrane. We credit Dr. Van Ness's opinion that adopting Bianchi's approach to treating cells with paraformaldehyde creates a means for cellular DNA to escape. Ex. 2078 ¶¶ 102–104; Ex. 2101, 106:4–19 (testimony of Dr. Edwards agreeing that "DNA is one thing that can escape a cell when you permeabilize it"). Although Bianchi discloses a desire to keep "substantially all" of the target molecules inside the cells, by Bianchi's own definition, "substantially all" includes leakage of up to 50% of the target molecules and cellular DNA.

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[27] When asked whether he considered "any differences between paraformaldehyde and formaldehyde that might be relevant to DNA damage in forming your opinions," Dr. Edwards testified "[t]here should be no differences" and that "[i]n this field, these terms are often used interchangeably." Ex. 2299, 69:3–20. We find, therefore, that the issues, including DNA damage, with formaldehyde would have been understood to also apply to paraformaldehyde on this record.

Ex. 1004, 1:42–49; Ex. 2078 ¶¶ 106–108. We find that increasing potential leakage of DNA from the maternal cells runs counter to Chiu's objectives. As Dr. Edwards concedes, "DNA leaking out of cells" is something "Chiu tells us you do not want it to happen." Ex. 2101, 107, 21–25.

Petitioner contends that its modification of Chiu relies only on Bianchi's alleged "stabilization" step. Pet. Reply 19 (arguing that Bianchi "included a permeabilization step separate from the fixation step") (citing Ex. 1045, 171:3–11 (testimony of Dr. Van Ness that he has performed assays with permeabilization and fixation steps)). We agree with Patent Owner, however, that Bianchi does not describe using paraformaldehyde in any separate stabilization or fixing step. PO Sur-Reply 7–8. Instead, Bianchi teaches a "permeabilization method" where the cell preparation is first incubated in paraformaldehyde and "***then*** is incubated ***permeabilized***" in a solution containing alcohol. Ex. 1004, 3:36–44 (emphasis added); Ex. 2078 ¶¶ 102–103 (testimony of Dr. Van Ness discussing Bianchi's permeabilization process). This suggests the paraformaldehyde incubation contributes, to at least some degree, to permeabilizing cells. And, even if the POSA would understand that paraformaldehyde provides some cell-stabilization function, Dr. Edwards admits that adding formaldehyde or paraformaldehyde will likely create "holes" and "gaps" in the cell membrane—even as the membrane becomes more rigid. Ex. 2101, 38:11–39:20 (testifying "there will probably be holes in the membrane when you add it to a cell" because "[i]t's a pretty harsh treatment on cells" and "my guess is that it would impact the entire fluidity of the membrane"), 103:15–20 (testifying that, as in Bianchi, "using a fixative to basically probably

rigidify, if you will, the cell, which will cause gaps in the membrane in some ways probably").

Returning to DNA leakage, we acknowledge that Bianchi prefers that greater amounts of DNA stay in the cells. *See* Ex. 1004, 1:42–48 (disclosing a range: a lower bound of "preferably 50%" and an upper bound of "more preferably 95" and "most preferably 99% or greater" of "the DNA of the cell remain in the cell"). But we credit Dr. Van Ness's unrebutted testimony that Bianchi does not disclose how the POSA could adjust its techniques so the cells may leak at greater or lesser rates, and that a POSA would understand the probability of obtaining a leakage rate of 1% is low. Ex. 2078 ¶¶ 100–102. Assuming the improbable occurred, and only about 1% of the DNA escaped, Dr. Van Ness testifies persuasively that a "POSA would realize that releasing 1% of cellular DNA in a sample in Chiu would have a negative effect on Chiu's fetal cell-free DNA analyses." *Id.* ¶¶ 107–108 (citing Dr. Edwards's testimony (Ex. 2101, 113:23–114:5): Q "[D]id you consider what effect leaking 1 percent of the cellular DNA in samples in Chiu would have on Chiu's free fetal DNA percentages? A[:] Well, leaking any is going to cause the fetal fraction to go down.")). Indeed, Dr. Edwards admits that "any DNA escaping from cells in Chiu can lower the free fetal fraction in a sample"—contrary to Chiu's goals. Ex. 2101, 107:21–108:11. Petitioner vaguely invokes "general knowledge" in response, and contends that Bianchi's "fixation" step is the only important disclosure (discussed

above). Pet. Reply 17–19.[28] Petitioner does not, however, provide persuasive argument or evidence to explain why creating holes in the cell membranes—possibly releasing 1% or more of the maternal DNA—would have been seen by the POSA as acceptable in the Chiu/Lo modified method.

On this record, we find the DNA leakage that would have been expected from adding paraformaldehyde undermines Petitioner's position that a POSA would have been motivated to modify Chiu/Lo as proposed.

We also agree with Patent Owner that Petitioner has not established that the POSA would have been motivated to add paraformaldehyde to the modified blood sampling and cffDNA-detection method of Chiu/Lo because the current record supports a connection between formaldehyde's use and DNA damage. On balance, we are persuaded on this record that the POSA would have had significant, unresolved concerns with introducing formaldehyde, and its potential to adversely affect cffDNA in the modified method of Chiu/Lo, undermining Petitioner's challenge.

Cellular DNA and cell-free DNA are not the same thing. Dr. Edwards confirms this—explaining that they are "quite different," and that the list of

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[28] Dr. Edwards's Supplemental Declaration devotes five paragraphs to a response on the issue of motivation to combine (covering all grounds). Ex. 1047 ¶¶ 14–18. Much of that testimony relates to a purported need to stabilize and ship samples to central labs and a known use of formaldehyde as a *cellular* fixative (with no apparent suggested or reported use with cell-free DNA). *Id.* We have considered this testimony, but find that it provides no direct or persuasive response to Dr. Van Ness's testimony, which we credit on this record, about the concerns with leaking even small proportions of maternal DNA in a cffDNA detection method under Petitioner's modification of the art. Ex. 2078 ¶¶ 102–108.

differences is "quite long." Ex. 2299, 29:7–30:22. It is not simply the location of the DNA as intracellular versus extracellular; there are also structural and biochemical differences. For example, cell-free DNA are short fragments compared to cellular DNA which is typically long, genomic DNA. *Id.* (noting the scale of the size difference with cell-free DNA typically "very small, on the order of a hundred base pairs" whereas cellular DNA may be "hundreds of millions of bases long"). There are also differences in methylation patterns and binding proteins—cellular DNA is predominantly intact, wrapped around proteins forming chromatin (a DNA/protein complex). *Id.*; *see also id.* at 32:11–16 (cell-free DNA is comparatively protein free (the DNA/protein complex form is eliminated)); Ex. 2101, 101:2–12. Patent Owner cites evidence on additional differences that are alleged to create obstacles to cffDNA recovery, including that it is available in limited quantities, can be diluted due to lysis, and is "easily damaged." Ex. 2086 (Hunsley Decl.) ¶ 4. Dr. Edwards agreed that cffDNA is small, available in limited quantities, and diluted by lysis, and "agree[d] it can be damaged" but demurred, "I don't know if I agree with the easily part." Ex. 2299, 158:19–161:22.

A key question presented in this case is whether a POSA would have been concerned with formaldehyde's potential effects on DNA, and cell-free fetal DNA in particular. Petitioner argues that a POSA would have been motivated to try "other means for reducing [maternal DNA] background," including adding paraformaldehyde to the method of Chiu/Lo, but the Petition does little to address whether that addition would have been

expected to adversely affect cell-free DNA, much less cffDNA.[29] Pet. 28. Bianchi does not speak specifically to that issue because, as Dr. Edwards admits, the DNA mentioned in Bianchi is "cellular" DNA and whatever DNA escapes the cells is just washed away. Ex. 2299, 59:16–19; Ex. 1004, 6:1–14); Ex. 2078 ¶¶ 101, 110. And, although Dr. Edwards concedes that it was known that formaldehyde can damage DNA—and the record includes evidence cautioning against the use of formaldehyde-containing compounds, even for applications that involved *cellular* DNA—Dr. Edwards admitted that, for his initial analysis, he "didn't consider whether the negative impacts of formaldehyde might have led someone in 2002 to pick a different chemical instead." Ex. 2101, 68:3–17; Ex. 2299, 57:23–58:4 ("[Y]ou don't dispute that formaldehyde has the potential to damage DNA including cell-free DNA, right? A[:] That is correct, It has the potential."). Petitioner's evidence in support of its alleged motivation to add paraformaldehyde is deficient on this record.

Patent Owner responded with evidence that a POSA would have been concerned about the detrimental effects of formaldehyde on nucleic acids. Srinivasan, for example, notes that attempts to extract usable DNA from formaldehyde fixed tissues have only been "variably successful," explaining

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[29] Petitioner initially suggested that paraformaldehyde might "replace" EDTA in Chiu's samples and method. Pet. 28. It is unclear why a POSA would have thought that paraformaldehyde might replace EDTA, the anticoagulant in Chiu (and Lo). Petitioner directs us to no evidence showing paraformaldehyde or formaldehyde were considered alternatives to an anticoagulant, or suggesting plasma samples (as in Chiu or Lo) could be prepared without an anticoagulant.

that "considerable evidence suggests that formaldehyde induces DNA degradation" with "few" studies reporting yield of high-molecular weight DNA. Ex. 2150, 1964 (disclosing "formaldehyde initiates DNA denaturation" and a high frequency of "sequence alteration"). Srinivasan further indicates that "the problems of formaldehyde" might be overcome by "use [of] an alternative fixative that is better suited for the preservation of nucleic acids." *Id.* at 1966. Patent Owner also cites Swenberg, as it reports on the problems with using formaldehyde, even with cellular applications and cellular DNA. PO Resp. 21–22 (citing Ex. 2139, 1 (identifying "DNA breaks" among other problems)). We credit Dr. Van Ness's opinion that such disclosures in the literature would have dissuaded a POSA from using formaldehyde or paraformaldehyde in the Chiu/Lo modified method. Ex. 2078 ¶¶ 114–115. That is especially so here, where we have a dearth of evidence suggesting formaldehyde's use in a sample where *cell-free* DNA is the analyte, and no sufficient, persuasive evidence or technical reasoning to explain why a POSA would not have been concerned with potential damage to the cffDNA.

In its Reply, Petitioner ignores Swenberg and embraces Srinivasan's alleged "criteria" for "the use of formaldehyde as a nucleic acid fixative." Pet. Reply 20 (citing Ex. 1051, 1965). Insofar as Petitioner suggests that Srinivasan discloses criteria for the use of formaldehyde to fix *free* nucleic acids, Petitioner is incorrect. It does not. The portion of Srinivasan cited by Petitioner relates to use as "***tissue*** nucleic acid fixative"—in other words, *cellular* nucleic acids. Ex. 1051, 1965 (emphasis added). Petitioner's expert admits that Srinivasan does not discuss cell-free DNA or cffDNA. Ex. 2299,

41:15–25 ("Q[:] Is it your understanding that the Srivastan [sic] paper discusses cell-free DNA at all? A[:] ***No just DNA damage due to fixation***.") (emphasis added)). On balance here, we find that Srinivasan is not specific to cell-free DNA and otherwise discourages formaldehyde's use due to potential harms to DNA more generally.

Petitioner also never explains how the cited "criteria" in Srinivasan align with Petitioner's proffered modification to Chiu/Lo. As discussed above, Petitioner purports to modify the Chiu/Lo method so that the cells are stabilized with paraformaldehyde for relatively long time periods—more than 24 to 48 hours to enable shipping off-site to a central lab. *See supra* Section II(E)(1)(c). That theory stands in tension with Srinivasan's disclosure that duration of tissue fixation should not exceed 3–6 hours. Ex. 1051, 1965; *see* Pet. Reply 20 (arguing a POSA would know that "***long durations*** or high concentrations of formaldehyde ***should be avoided***, [but] samples promptly processed and fixed yielded reliable nucleic acid analysis" (citing Ex. 1047 ¶ 18 (citing Srinivasan's 3–6 hour fixation time)) (emphasis added). Petitioner has not reconciled Srinivasan's disclosures with Petitioner's theory for modifying Chiu, Lo, and Bianchi, which theory presumes a long and sustained exposure of the maternal blood sample to paraformaldehyde.

When asked at the oral hearing how its theory was compatible with criteria indicating exposure of no more than 3–6 hours, Petitioner responded that there was an alleged "need for formaldehyde donors and quenchers, which is actually what's been adopted in the industry." Tr. 20:22–21:14 (identifying "Cyto-Chex and Rao reference," as well as the '517 patent

(Ex. 1049)). If Petitioner wanted to assert a combination based on Chiu and Streck's '517 patent, it could have made that argument in the Petition. It did not. Petitioner also never proposed as part of its theory in any paper that a POSA would have further added "quenchers" to its combination of Chiu and Bianchi. The oral hearing is too late. (At best, Petitioner's response at oral argument citing Cyto-Chex and Rao relates to its second ground, which we address below.) When pressed on the fact that Petitioner's theory requires cffDNA undergo "sustained exposure to whatever the stabilizer is," counsel shifted again, noting "different variables . . . that you could use, you could decrease the concentration of formaldehyde." *Id.* at 22:7–19. This too is not explained sufficiently in Petitioner's papers. It is of the "utmost importance" that a petitioner identify with particularity its theories and supporting evidence in the petition itself. *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1369 (Fed. Cir. 2016) (explaining that such particularity is required by statute under 35 U.S.C. § 312(a)(3)).

Finally, we agree with Patent Owner that, inasmuch as Petitioner is suggesting a POSA might simply "tailor" the processing conditions for using formaldehyde effectively, Petitioner's argument fails. PO Sur-Reply 9–10. As Patent Owner explains persuasively, Petitioner leaves it "entirely unclear" what those specific conditions are and how they would be modified to render formaldehyde safe for use with Chiu's and Lo's cffDNA. *Id.* Petitioner's suggestion is also at odds with testimony from its expert in the parallel litigation. *Id.* at 10 (citing Ex. 2300). Indeed, in that proceeding, Dr. Larry Dumont testified that "formaldehyde . . . ha[s] been demonstrated to degrade nucleic acids," that "low concentrations of formaldehyde . . .

significantly inhibit[] (or eliminates altogether) the ability to successfully perform PCR amplification on DNA," and that "literature suggests that these 'agents' would not be compatible with standard methods used to detect and analyze DNA.")). Ex. 2300 ¶ 390.[30] Petitioner has not shown sufficiently, on this record, that a POSA would have understood that paraformaldehyde could be used effectively with the Chiu/Lo cffDNA. The evidence presented by Patent Owner undermines Petitioner's assertion that a POSA would have been motivated to modify Chiu/Lo as proposed.

Altogether, considering the argument and evidence presented through trial, Petitioner does not persuade us that a POSA would have been motivated to combine and modify Chiu, Lo, and Bianchi in the manner proposed to arrive at the subject matter of claim 55.[31]

4. Dependent claims 82–91, 94–96 and 133

Claims 56–63, 66–69, 80, and 127–132 all depend from claim 55. For these dependent claims, Patent Owner raises the same arguments as addressed above. PO Resp. 8 (argument for "All Claims"). Petitioner's challenge to the dependent claims relies on its claim 55 analysis (*see, e.g.*, Pet. 29–33), and Petitioner does not argue or show that its challenge to the dependent claims makes up for deficiencies we have noted above. Pet.

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[30] It appears Dr. Dumont's testimony related, in particular, to whether certain of the '277 patent's claims were enabled. *See, e.g.*, Ex. 2300 ¶ 369.

[31] We need not reach Patent Owner's argument on secondary considerations of nonobviousness here. *See Hamilton Beach Brands, Inc. v. f'real Foods, LLC*, 908 F.3d 1328, 1343 (Fed. Cir. 2018) (holding that there is no need to reach objective indicia of nonobviousness where the petitioner has not made a showing necessary to prevail on threshold obviousness issues).

Reply 14–21 (same responsive argument for all claims); *see supra* Section II(E)(3). Thus, we conclude that Petitioner has not proved that claims 56–63, 66–69, 80, and 127–132 are unpatentable based on the combination of Chiu, Lo, and Bianchi. *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992) ("[D]ependent claims are nonobvious if the independent claims from which they depend are nonobvious.").

F. Obviousness over Chiu, Lo, and Rao

Petitioner asserts that claims 55–63, 66–69, 80, and 127–132 would have been obvious over Chiu, Lo, and Rao. Pet. 41–60; *see id.* at 41–48 (claim 55). Chiu, Lo, and Rao are discussed above. *See* Section II(D)(1), (3), (4). Petitioner's theory on Chiu, Lo, and Rao is substantially similar to the Chiu/Lo/Bianchi challenge. Petitioner relies on the same teachings in Chiu and Lo as disclosing methods for processing blood samples and isolating and analyzing cffDNA from maternal plasma to determine a sequence of a locus of interest on such DNA. *Id.* at 41–43. As with the grounds above, Petitioner contends that Chiu and Lo do not teach a lysis-inhibiting "agent" as claimed and, thus, Petitioner turns to Rao in a similar way to Bianchi. *Id.* at 44–45. Petitioner contends that a POSA "would have been motivated to try alternative or additional agents to EDTA to reduce cell lysis and to avoid a filtration step or an extra centrifugation step as suggested by Chiu to remove maternal cells and cellular debris" and, like it did with Bianchi, Petitioner contends that Rao discloses "an alternative" by teaching cell-stabilizing compounds that may stabilize rare cells. *Id.* at 45–47 (citing Ex. 1005, Abstr.; Ex. 1002 ¶¶ 30, 37, 51–52). According to Petitioner, Rao

identifies commercial stabilizers like "Cyto-Chex™ from Streck Laboratories," a "formaldehyde donor" like "imidazolidinyl urea . . . [or] paraformaldehyde," or an "aldehyde" like "formaldehyde." *Id.* at 45.

Petitioner's motivation to combine and reasonable expectation of success positions largely mirror its argument on Chiu/Lo/Bianchi. *Id.* at 45–48. Petitioner contends a POSA would have been motivated to try other means to reduce maternal DNA background, including adding agents to inhibit cell lysis, and to determine whether such agents provide advantages over Chiu's use of EDTA with filtration or microcentrifugation step and to possibly eliminate those "extra steps." *Id.* at 47 (citing Ex. 1002 ¶ 51); *see also id.* (arguing a POSA would have been motivated to add agents "such as the agents described in Rao" to reduce apoptosis during storage and possibly simplify Chiu's processing steps "(i.e., by eliminating a second centrifugation or filtration step)"). According to Petitioner, a POSA "would have had a reasonable expectation that use of an aldehyde, or other cell lysis inhibitor disclosed in Rao, in place of or in addition to the EDTA and second centrifugation and/or filtration steps in Chiu's methods, would have been successful for detecting fetal nucleic acids in a sample as both techniques inhibit cell lysis and would have been expected to reduce assay background noise created by lysed maternal cells." *Id.* at 47–48 (citing Ex. 1002 ¶ 52).

Petitioner expounds on its motivation theory as discussed above (*supra* Section II(E)(1)(c)), and, although Petitioner's assertions in the Petition, noted immediately above, refer somewhat vaguely to adding Rao's alleged "agents" to Chiu/Lo, Petitioner confirms that its theory is based on

the addition of formaldehyde or formaldehyde donors, not other agents in Rao. Tr. 22:24–23:17.[32]

Based on Petitioner's overlapping argument, Patent Owner's argument against the combination of Chiu/Lo/Rao tracks its argument about Chiu/Lo/Bianchi, discussed above. PO Resp. 38–48.[33] For example, Patent Owner contends that Rao's cell-detection approach adds agents that "creat[e] ***more permeable*** cells that allow particles to travel through their membrane, thus ***increasing*** the potential release of DNA from cells." *Id.* at 43–44 (citing Ex. 1005, 3:21–25, 12:26–30, 13:16–23; Ex. 2078 ¶ 144 (testimony of Dr. Van Ness that applying Rao's teachings would increase the risk of releasing DNA and increasing DNA background)). Patent Owner also argues, like it did with Bianchi, that Rao is unrelated to detection or analysis

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[32] Petitioner has argued that Cyto-Chex blood collection tubes "contain[] EDTA and a formaldehyde donor (imidazoli[di]nyl urea)." Pet. Reply 12 (citing Ex. 1050, 9:12–17); *see also* Ex. 2086 ¶ 6 (testimony of Brad Hunsley, Streck Inc.'s Director of Research & Development that Streck's Cyto-Chex blood-collection tubes have been available for sale since about 2003, and that such products are the subject of US Patent Application No. 10/605,669 (which appears to be the application filed October 16, 2003, that issued April 6, 2021, as US 10,966,421 (Ex. 1050)). For purposes of this decision, we will treat that characterization of Cyto-Chex by Petitioner as accurate and that it includes a formaldehyde donor. We note, however, that Rao does not explicitly list Cyto-Chex's components.

[33] Patent Owner disputed the prior-art status of Rao for dependent claims 60 and 132. PO Sur-Reply 14–18. Petitioner, conversely, alleged that such claims do not have an effective date before August 29, 2003, and, if they did, Rao would still be prior art based on Rao's provisional application filing date in August 2001. Pet Reply 4–13. We need not decide this issue to resolve the case because, even if Rao is prior art for all claims, Petitioner's challenge based on Chiu/Lo/Rao fails for other reasons, as discussed herein.

of cell-free DNA; to the extent DNA is mentioned in Rao, Patent Owner argues Rao describes concerns with formaldehyde, including its negative impacts on DNA. *Id.* at 45–46; Ex. 2078 ¶¶ 145–146 (Dr. Van Ness explaining, *inter alia*, that Rao discards the extracellular fraction of its samples), 147 (opining, e.g., that Rao discloses that formaldehyde released from formaldehyde donors was known to "irreversibly cross link[] nucleic acids," citing Ex. 1005, 4:8–11); PO Sur-Reply 19–20 (citing Ex. 2299, 146:11–147:21 (admissions of Dr. Edwards that Rao does not analyze plasma or DNA, or determine whether its stabilizers had caused harm to DNA or rendered it unsuitable for assay methods like PCR)).

Continuing, Patent Owner argues, "Petitioner's focus on formaldehyde for its Chiu/Lo/Rao combination suffers from the same deficiency as its Chiu/Lo/Bianchi combination." PO Resp. 46. Like it argued above, Patent Owner contends Petitioner fails to address adequately the dangers of formaldehyde and its known potential to damage nucleic acid products. *Id.* at 46–48 (citing Ex. 2078 ¶¶ 149–154; cross-referencing its argument and evidence contra the Chiu/Lo/Bianchi combination). According to Patent Owner, as with publications such as Srinivasan, "Rao itself would have dissuaded a POSA from using formaldehyde" because of its noted problems and deficiencies. *Id.* at 47 (citing, e.g., Ex. 2150, 1966 (recommending alternative fixatives even for cellular applications); Ex. 1005, 4:8–13 (Rao disclosing that formaldehyde from donors is reported to react with nucleic acid bases and irreversibly cross-link DNA); Ex. 2101,

150:4–16 (testimony of Dr. Edwards that formaldehyde will "cause cross-links to DNA")).[34]

Rather than substantially repeat the analysis and evidence discussed above (*supra* Section II(E)(3)-(4)), we cross-reference and adopt that discussion here. In summary, we find that Petitioner has not proved by a preponderance of the evidence that a POSA would have added formaldehyde or a formaldehyde donor from Rao to the modified method of Chiu/Lo. As explained above, we find on this record that a POSA would have been discouraged from adding reagents that would create holes in the cells because that provides a route for DNA to escape and add undesired maternal background DNA. Dr. Edwards admits that formaldehyde will "create holes in membranes" and characterizes formaldehyde as "a pretty harsh treatment on cells . . . when you do this." Ex. 2101, 39:8–15; *see also id.* at 107:22–25 (testifying that DNA leaking from cells is something "Chiu tells us you do not want it to happen"); Ex. 2078 ¶ 144 (testifying that Rao's treatment of cells makes them more permeable).

Even if the DNA leakage issue would not have caused the POSA to turn away from introducing formaldehyde, we credit Patent Owner's rebuttal evidence showing that formaldehyde was known to cause problems, including DNA breaks and degradation, even relative to *cellular* applications

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[34] Petitioner's and Patent Owner's argument in the respective Reply and Sur-Reply about a motivation to combine Chiu/Lo/Rao are also substantially the same as argued for Chiu/Lo/Bianchi. Pet. Reply 14–21 (arguing all grounds together); PO Sur-Reply 18–20 (cross-referencing Chiu/Lo/Bianchi argument).

and *cellular* DNA (as opposed to cell-free DNA, where formaldehyde's effects were previously unknown and unreported). *See, e.g.*, Ex. 2150, 1964–1966. We also credit Dr. Van Ness's opinion that concerns with formaldehyde would, on balance, have discouraged the POSA from using it in the context of cffDNA analysis. Ex. 2078 ¶¶ 148–154. Such evidence belies Petitioner's assertion that a POSA would have been motivated to add formaldehyde or formaldehyde-releasing donor compounds to the method of Chiu/Lo.

Even Rao discloses that "[f]ormaldehyde released from these so-called formaldehyde donors has been reported to react with nucleic acid bases, particularly adenine, to reversibly form hydroxymethylol derivatives and methylene bridges thereby ***irreversibly crosslinking*** nucleic acids." Ex. 1005, 4–11 (emphasis added); Ex. 2078 ¶ 147 (testimony of Dr. Van Ness that such cross-linking would be detrimental for nucleic-acid applications). Dr. Edwards admits that adding formaldehyde "could induce cross-links, and they need to be reversed" in order to amplify cffDNA for analysis. Ex. 2101, 150:3–151:2 (admitting formaldehyde's addition will "cause cross-links to DNA").[35] How the "irreversible" DNA crosslinks of the sort described in Rao would be reversed, or why that would (or would not) be a concern, is left unaddressed by Petitioner. Pet. Reply 14–21 (not addressing this argument); Ex. 2078 ¶¶ 14–18 (rebuttal testimony of

[35] *See* Ex. 2101, 72:17–22 ("Q[:] But could cross-links on DNA affect one's ability to amplify or detect the DNA? A[:] Well, one would need to reverse the cross-links, and then they should -- one should be able to amplify or detect the DNA.").

Dr. Edwards on motivation-to-combine issues, not addressing Rao's disclosure of irreversible crosslinking of DNA by formaldehyde donors).

Petitioner identified Cyto-Chex as a stabilizer disclosed in Rao, yet Cyto-Chex still involves a formaldehyde donor. Pet Reply 12 (asserting that Cyto-Chex includes the formaldehyde donor imidazolidinyl urea); *see supra* n.32. There is no dispute on this record that formaldehyde donors will still release formaldehyde. *See, e.g.*, Ex. 1005, 3:26–4:20 (describing the release of formaldehyde by such donors). And, as such, we find here that similar concerns to those above would have discouraged the POSA's use of Cyto-Chex in a wholly new way involving cffDNA.

During the oral hearing, Petitioner suggested for the first time that a POSA may have combined "formaldehyde donors and quenchers" to avoid damage from sustained exposure of cell-free DNA to formaldehyde. Tr. 20:22–21:14 (asserting that such combination is "actually what's been adopted in the industry" and "that really gets us back to the Cyto-Chex and Rao reference," and further "Cyto-Chex, according to the '517 patent, can include a formaldehyde donor and a formaldehyde quencher"). As noted above, Petitioner never asserted in any pre-hearing paper that "quenchers" would be added in its combined prior art. Moreover, Streck's '517 patent (Ex. 1049) was not cited in the Petition. And, although it is cited in Petitioner's Reply, Petitioner did not identify alleged teachings about "quenchers" to argue that a POSA would have selected such compounds for

further addition in its Chiu/Lo/Rao combination.[36] Pet. Reply 17–20. On this record, we find that Petitioner forfeited the argument that the POSA would have added formaldehyde-quenching compounds to its combination based on Chiu and Rao. Paper 44 (Order setting oral hearing), 4 (citing, e.g., *Dell Inc. v. Acceleron, LLC*, 884 F.3d 1364, 1369 (Fed. Cir. 2018) (holding that the Board is obligated under its own regulations to dismiss untimely argument "raised for the first time during oral argument")).

Even if we were to consider Petitioner's untimely argument, Petitioner makes no persuasive showing that Streck's '517 patent relates to, or suggests any use of, formaldehyde donors with cell-free DNA or cffDNA. And, absent evidence tying the alleged industry practices to what a POSA would have done at the time the '277 patent was filed, Petitioner's assertion that what the industry has actually done is use formaldehyde donors and quencher compounds, even if true, does not weigh in favor of a determination of obviousness on this record. Moreover, the record here

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[36] We recognize that Rao lists the '517 patent among at least seven other Streck patents identified as disclosing stabilizing agents. Ex. 1005, 3:33–4:3 (listing "US 5,849,517" among others). But it is not apparent, and Petitioner never argued, that the '517 patent is incorporated by reference into Rao. Assuming arguendo that the '517 patent is in some manner related to Cyto-Chex, the mere disclosure of the '517 patent in Rao does little to further Petitioner's case, especially absent timely argument and evidence about the alleged obviousness of adding quenching compounds. In any event, there is evidence of record that the '517 patent is not related to Cyto-Chex. Streck's Director of R&D testified that another product called "Streck Cell Preserve" is the subject of the '517 patent. Ex. 2086 ¶ 6; *see also id.* ¶ 7 (testifying that Cyto-Chex BCTs (blood collection tubes) are the subject of another patent application (which is not identified in Rao)).

suggests that any industry adoption of such compounds for use with cffDNA analysis occurred only *several years after* the filing (in 2003) and publication (in 2004) of the application that matured into the challenged '277 patent. Ex. 1001, codes (22), (65)); Ex. 2109, cover page (U.S. Patent 9,926,590, assigned to Streck, Inc., titled "Devices and Compositions for Preservation of Cell-Free Nucleic Acids" claiming priority through many applications, the earliest of which are in 2009 and 2010), 13:20–45 (claim 1); Ex. 2108, 1 (Streck website listing U.S. Patent 9,926,590 as covering its "Cell-Free DNA BCT®"); Ex. 2086 ¶¶ 6–8 (testimony of Mr. Hunsley that, prior to 2010, "there is no literature to suggest use of Streck Cell Preserve or Cyto-Chex® BCT for use in fetal DNA recovery"). PO Sur-Reply 22 (citing Exs. 2108, 2109, 2086).

For the reasons above, we conclude that Petitioner has not proved by a preponderance of the evidence on this record that claim 55 and its dependent claims challenged here are unpatentable as obvious over Chiu, Lo, and Rao.

III. MOTIONS FOR PROTECTIVE ORDER AND TO SEAL

Patent Owner moves for entry of a stipulated protective order and for an order sealing portions of the Patent Owner Response (Paper 19), all of Exhibits 2170–2173, and portions of Exhibit 2080. *See* Paper 19 (attaching stipulated protective order as Appendix A). That motion is unopposed.

A party may move to seal confidential information including, *inter alia*, sensitive commercial information. Consolidated Patent Office Trial Practice Guide, 19 (Nov. 2019); 37 C.F.R. § 42.54. It is the movant's burden to show good cause for sealing such information, and we balance the party's asserted need for confidentiality with the strong public interest in

open proceedings. *Argentum Pharms. LLC v. Alcon Research, Ltd.*, IPR2017-01053, Paper 27 at 4 (PTAB Jan. 19, 2018) (informative).

Patent Owner provides a sufficient explanation for sealing the relevant portions of the Patent Owner Response and the identified exhibits. Exhibits 2170–2173 are license or settlement agreements between Patent Owner and third parties setting forth, for example, payment terms and sales data. Patent Owner states that it produced those agreements with the permission of third parties on the condition that the agreements remain sealed. Paper 19, 3–4. The subject portions of the Patent Owner Response and Exhibit 2080 include discussions about those agreements. Patent Owner has also provided redacted versions of Exhibit 2080 and the Patent Owner Response (Paper 20) so the record may remain clear and reasonably open.

Patent Owner has established good cause for sealing Exhibits 2170–2173, portions of Ex. 2080, and the Patent Owner Response. The stipulated Protective Order includes minor changes from our default language. That Protective Order, Appendix A to Paper 19, is entered.

Petitioner also filed a Motion to Seal (Paper 30). Petitioner contends that portions of its Reply (Paper 29), and portions of Exhibit 1054 (deposition transcript of Paul K. Meyer) should be sealed because those papers include information that Patent Owner considers contain confidential business information (e.g., licensing practices). Paper 30, 1. Petitioner has also filed public redacted versions of the Petitioner Reply (Paper 31) and Exhibit 1054. For the same reasons discussed above regarding Patent Owner's Motion to Seal, Petitioner has shown good cause for granting its motion. Petitioner's Reply (Paper 30) and Exhibit 1054 are sealed.

IV. MOTION TO EXCLUDE

Petitioner moves to exclude Exhibit 2301, a deposition transcript of Galla Chandra Rao ("Rao Transcript") taken in connection with the lawsuit between Patent Owner and third party Quest Diagnostics Incorporated. *See supra* Section I(A) (listing related matters). Mot. to Exclude 1–3. Petitioner contends that the Rao Transcript should be excluded as inadmissible hearsay and as irrelevant. Mot. to Exclude 1–3 (citing Fed. R. Evid. 802, 401, 402, and 403).

Because we do not rely on the Rao Transcript in making our determinations in this Final Written Decision, Petitioner's Motion to Exclude is moot and, accordingly, dismissed.

V. CONCLUSION

In Summary:

Claims	35 U.S.C. §	Reference(s)/Basis	Claims Shown Unpatentable	Claims Not shown Unpatentable
55–63, 66–69, 80, 127–132	103(a)	Chiu, Lo, Bianchi		55–63, 66–69, 80, 127–132
55–63, 66–69, 80, 127–132	103(a)	Chiu, Lo, Rao		55–63, 66–69, 80, 127–132
Overall Outcome				55–63, 66–69, 80, 127–132

VI. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that the Petitioner has not proved by a preponderance of the evidence that claims 55–63, 66–69, 80, and 127–132 are unpatentable;

FURTHER ORDERED that Patent Owner's Motion for Entry of Stipulated Protective Order (Appendix A to Paper 19) and to Seal is *granted*;

FURTHER ORDERED that Petitioner's Motion to Seal (Paper 30) is *granted*;

FURTHER ORDERED that Petitioner's Motion to Exclude (Paper 42) is *dismissed* as moot; and

FURTHER ORDERED that, because this is a Final Written Decision, parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.