IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Fujirebio Diagnostics, Inc.
Petitioner

v.

Quanterix Corp.
Patent Owner.

U.S. Patent No. 11,275,092
Issue Date: March 15, 2022

Title:
Methods of determining a treatment protocol for
and/or a prognosis of a patient's recovery from a brain injury

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Inter Partes Review No.: IPR2025-01060

DECLARATION OF JOHN TODD PH.D IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 11,275,092

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

II. QUALIFICATIONS 3

III. RELEVANT LEGAL STANDARDS 8

IV. OVERVIEW 12

V. BACKGROUND 14

A. Summary of the '092 Patent 14

B. Prosecution History 16

C. Priority Date of the Challenged Claims 18

D. Claim Construction 19

E. Person of Ordinary Skill in the Art 19

F. State of the Art 20

1. The Role of Tau in Alzheimer's Disease and Related Neurological Disorders 20

2. SMA and Detecting Tau 27

VI. IDENTIFICATION OF HOW THE CHALLENGED CLAIMS ARE UNPATENTABLE 37

A. Ground I: Rissin in view of Todd 37

1. The Rissin Reference 37

2. The Todd Reference 40

3. The Motivation to Combine the Teachings of Rissin in view of Todd 43

4. Detailed Application of Rissin in view of Todd 47

B. Challenge 2: Claims 1-21 of the ’092 Patent are invalid as obvious over Rissin in view of Voorheis 66

1. The Motivation to Combine Rissin in view of Voorheis.....69

2. Detailed Application of Rissin in view of Voorheis.....72

CONCLUSION AND DECLARATION.....1

APPENDIX A 1

I, John Todd declare as follows:

I. INTRODUCTION

1. I am over the age of eighteen (18) and otherwise competent to make this Declaration.

2. I have been retained by Fujirebio Diagnostics, Inc. (Petitioner) as an independent, technical expert consultant in this proceeding before the United States Patent and Trademark Office (USPTO). I am being compensated at my rate of $375 per hour for my services. No part of my compensation is dependent on my opinions or on the outcome of this proceeding. I have no financial interest in any of the parties to this proceeding.

3. This Declaration is in support of the Petition for *inter partes* review involving U.S. Patent 11,275,092 (the "'092 Patent" or "the Patent") (EX-1001), entitled "METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND / OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY" and listing David Wilson, John Henrik Zetterberg, Kaj Blennow and Jeffrey D. Randall as the inventors.

4. For the purposes of this *inter partes* review as I discuss later, I have been instructed to assume that the effective filing date of the Claims of the '092 Patent challenged by the Petitioners in this *inter partes* review is no earlier than April 12, 2011.

5. I have been instructed to assume for purposes of this proceeding that Quanterix Corp.is the patent owner.

6. The '092 Patent is generally directed to a method for producing a patient bodily fluid sample having an analytically quantified amount of endogenous tau protein using an assay that is highly sensitive. I am familiar with the technology described in the '092 Patent as of the earliest possible priority date of April 12, 2011,.

7. In preparing this Declaration, I have reviewed the '092 Patent (EX-1001) and its prosecution history (EX-1002), as well as the patents and documents cited herein, and I have considered these documents in light of the general knowledge in the art as of April 12, 2011. In formulating my opinions, I have relied upon my experience in the relevant art. I have also considered the viewpoint of a person of ordinary skill in the art ("POSA") in the relevant field, as of April 12, 2011, who I describe below (§ V.D). For convenience, the materials I considered in arriving at my opinions are listed in Appendix A.

8. I have been asked to provide my technical expertise, analysis, insights and opinions regarding the '092 Patent and relevant references that form the basis of the arguments set forth in the accompanying Petition for *inter partes* review of the '092 Patent. As described in detail below, I offer the following opinions in this Declaration:

9. A POSA would have found Claims 1, 6-21 of the '092 Patent to be obvious over Rissin in view of Todd and a POSA would have been motivated to combine the teachings of these references.

10. A POSA would have found Claims 1, 6-21 of the '092 Patent to be obvious over Rissin in view of Voorheis and a POSA would have been motivated to combine the teachings of these references.

11. Unless otherwise indicated, the emphasis in quotations has been added.

II. QUALIFICATIONS

12. My qualifications for forming the opinions set forth in this declaration are summarized here and explained in more detail in my *curriculum vitae*, which is included as EX-1004. EX-1004 also includes a non-exhaustive list of my publications and patents.

13. I am currently Principal Consultant at JTConsulting (since 2019), where I advise diagnostic firms and investors on molecular and

protein biomarker assay development, regulatory strategy, and clinical validation.

14. I received a BS in Biology and a Ph.D. in Biochemistry in 1981 from the University of Southern California, with doctoral research focused on biochemical mechanisms relevant to clinical diagnostics, including immunoassay technologies.

15. From 1981 to 1984, I completed postdoctoral training in Cell and Molecular Biology at NYU under Dr. David Sabatini's Lab, specializing in protein targeting and membrane biochemistry.

16. At Enzo Biochem (1984–1987), I led development of DNA probe clinical diagnostics for infectious diseases such as HPV and CMV, foundational to later biomarker-based clinical assays.

17. As Group Leader at Baxter Healthcare's Pandex Division (1988–1991), I developed fluorogenic immunoassays for infectious disease applications in blood screening, gaining early experience in high-sensitivity, automated detection systems.

18. At Chiron/Bayer Diagnostics (1992–2000), I led product development and global clinical marketing for branched DNA assays measuring viral RNA for HIV and HCV, validating the predictive power of low-abundance biomarkers.

19. I co-authored landmark clinical trials (e.g., Mellors et al., Ann Intern Med, 1997; Todd et al., J Acquir Immune Defic Syndr, 1995, Mellors, J.W., et al., Science, 1996) that established viral load as a quantitative marker for prognosis and treatment guidance in patients infected with HIV.

20. From 2000 to 2004, I consulted on molecular diagnostic strategy at Halteres Associates and NuGEN Technologies, contributing to product roadmaps for RNA amplification and detection platforms.

21. As VP of R&D (2004–2013) and Chief Science Officer (2016–2019) at Singulex, I led development of the Single Molecule Counting (SMC™) immunoassay system, the technological foundation for the Simoa platform used in the ’092 patent.

22. I co-authored Todd et al., Clin Chem (2007), a pivotal study validating the quantification of low abundance biomarkers in blood plasma using femtomolar-level detection, and Todd et al., Drug Discovery World 2008, directly supporting the methods of U.S. Patent 11,275,092.

23. I managed development of clinical-grade assays for the ultrasensitive detection of cardiac troponin I, IL-6, TNF-alpha, IL-17A and other biomarkers, including design of serial sampling protocols to monitor

dynamic concentration profiles over time—an approach central to the ’092 patent’s claims.

24. My team developed over 50 single molecule assays to quantify proteins in blood plasma. We were able to transfer any immunoassay, without failure from a traditional non-sensitive ELISA platform, onto a single molecule counting platform resulting in assays with sub pg/mL limits of quantification. My team also discovered and published algorithms based upon ultrasensitive detection of cardiac troponin-I in blood plasma for early assessment of cardiac ischemia, forming the scientific basis for the prognostic stratification in the ’092 patent.

25. I oversaw assay validation trials across North America and Europe, confirming the reproducibility and clinical relevance of high-sensitivity protein detection in plasma and serum using ultrasensitive single molecule assays.

26. I hold or contributed to more than 30 U.S. and international patents, including those covering ultrasensitive immunoassay chemistry, bead-based single molecule immunoassays and clinical applications of these assays.

27. I was the first (date 2009) to develop ultrasensitive assays using single-molecule digital assays for measuring proteins associated with

neurological conditions (Tau, Amyloid β (Aβ or Abeta) 40 and 42) and demonstrated that Abeta amyloids 40 and 42 could be measured in human plasma despite the blood-brain barrier, a key challenge addressed by the ’092 patent.

28. I contributed to companion diagnostics programs with industry partners, helping to align biomarker thresholds with clinical decision-making pathways for neurodegenerative and cardiac conditions.

29. My expertise has been applied to Food & Drug Administration (FDA), Clinician Laboratory Improvement Amendment (CLIA), and CE-IVD regulatory filings (*i.e.*, a CE marking that is required for all *in vitro* diagnostics (IVD) sold in Europe), particularly in the context of using biomarkers for early diagnosis and treatment monitoring following acute hypoxic events.

30. I co-authored multiple peer-reviewed publications and conference abstracts cited in the ’092 patent related to blood plasma protein quantification following cardiac arrest and hypoxia, including Wu et al. (Development and preliminary clinical validation of a high sensitivity assay for cardiac troponin using a capillary flow (single molecule) fluorescence detector. Clin Chem. Nov. 2006; 52(11):2157-9).

31. I have provided consulting services in IP strategy, especially involving ultrasensitive detection platforms and their clinical applications and helped manage the IP portfolio at Singulex, Inc.

III. RELEVANT LEGAL STANDARDS

32. I do not provide any legal opinions in this Declaration. I have been informed and understand that certain legal standards are to be applied by technical experts in forming opinions regarding the meaning and validity of patent claims. I have been asked to provide my opinions regarding whether the claims of the '092 Patent would have been obvious to a person having ordinary skill in the art at the time of the alleged invention, in light of the prior art.

33. Further, I have been informed and understand that a patent claim is not patentable as obvious (a requirement I understand to be governed by a statute, 35 U.S.C. § 103) if the differences between the patent claim and the prior art are such that the claimed subject matter as a whole would have been obvious at the time the claimed invention was made to a person having ordinary skill in the relevant art. Obviousness, as I have been informed and understand, is based on the scope and content of the prior art, the differences between the prior art and the claim, the level

of ordinary skill in the art, and, to the extent that they exist, certain objective indicia of non-obviousness.

34. I understand that objective indicia can be important evidence regarding whether a patent is obvious or nonobvious, if it has an appropriate nexus to the claimed invention, i.e., is a result of the merits of a claimed invention (rather than the result of design needs or market-pressure advertising or similar activities). Such indicia include: commercial success of products covered by the patent claims; a long-felt need for the invention; failed attempts by others to make the invention; copying of the invention by others in the field; unexpected results achieved by the invention as compared to the closest prior art; praise of the invention by the infringer or others in the field; the taking of licenses under the patent by others; expressions of surprise by experts and those skilled in the art at the making of the invention; the patentee proceeded contrary to the accepted wisdom of the prior art, and, the contemporaneous development of the subject matter claimed by others.

35. I have been informed that whether there are any relevant differences between the prior art and the claimed invention is to be analyzed from the view of a person of ordinary skill in the relevant art at the time of the invention. As such, my opinions below as to a person of

ordinary skill in the art are as of the time of the invention, even if not expressly stated as such; for example, even if stated in the present tense.

36. In analyzing the relevance of the differences between the claimed invention and the prior art, I have been informed that I must consider the impact, if any, of such differences on the obviousness or non-obviousness of the invention as a whole, not merely some portion of it. The person of ordinary skill faced with a problem is able to apply his or her experience and ability to solve the problem and also look to any available prior art to help solve the problem.

37. I have been informed that a precise teaching in the prior art directed to the subject matter of the claimed invention is not needed. I have been informed that one may take into account the inferences and creative steps that a person of ordinary skill in the art would have employed in reviewing the prior art at the time of the invention. For example, if the claimed invention combined elements known in the prior art and the combination yielded results that were predictable to a person of ordinary skill in the art at the time of the invention, then this evidence would make it more likely that the claim was obvious. On the other hand, if the combination of known elements yielded unexpected or unpredictable results, or if the prior art teaches away from combining the known

elements, then this evidence would make it more likely that the claim that successfully combined those elements was not obvious.

38. I have been informed and understand that there are recognized, exemplary, rationales for combining or modifying references to show obviousness of claimed subject matter. Some of the rationales include the following: combining prior art elements according to known methods to yield predictable results; simple substitution of one known element for another to yield predictable results; use of a known technique to improve a similar device (method or product) in the same way; applying a known technique to a known device (method or product) ready for improvement to yield predictable results; choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success; known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art; and some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art teachings to arrive at the claimed invention.

IV. OVERVIEW

39. The Challenged Claims are invalid as obvious under 35 U.S.C. § 103 over the prior art. The claims are directed to producing a patient bodily fluid sample having an analytically quantified amount of endogenous tau protein. The Challenged Claims recite three well-known, well-understood steps:

- **Step A:** obtaining a sample of blood or a blood component;
- **Step B:** diluting the sample; and
- **Step C:** quantifying the amount of tau protein in the diluted sample using an assay, wherein the assay is sensitive (limit of quantification <0.2 pg/ml) and the tau protein is present in a low concentration (less than about 5 pg/ml).

40. Step A (obtaining a sample) is required of virtually any blood-based immunoassay and had been performed for decades prior to the purported invention. There is nothing unique with respect to how the blood sample is obtained.

41. Steps B and C (diluting a sample and analyzing it) were also routine in the art. Dr. David Walt and Dr. David Duffy at Tufts University assisted in the publication of numerous articles that taught the claimed steps of "diluting" and "analyzing" a blood sample using ultra-sensitive analytical techniques, such as single molecule assays ("SMA" or

"SiMoA"). EX-1010 (diluting sample 1:4 before analyzing with SMA); EX-1011 (discussing Quanterix's SMA approach); EX-1016 (disclosing proof-of-concept for Quanterix's SMA). So too did the several other groups, including me and my colleagues at Singulex, Inc., Mirkin *et al*. at Northwestern University, Nalefski, *et al*. at U.S. Genomics, and the PO itself. EX-1012, 1990; EX-1013; EX-1014; EX-1035; EX-1015. By April 2011, analytical techniques using SMAs had pushed the detection limit of proteins in bodily fluids into the attomolar range (*i.e.*, $1x10^{-18}$ mol/L) or sub pg/mL range, and were widely understood to "provide[] a promising approach for measuring extremely low concentrations of proteins." EX-1005, 595; EX-1015, 90.

42. Though these SMA techniques were well-known since the early 2000's, the provisional applications that led to the '092 patent were not filed until April and August 2011. EX-1001; EX-1002. Any incremental improvements in the '092 patent as to SMA developed by Drs. Walt and Duffy were prior published, and the '092 patent simply provides conventional SMA applied to measure endogenous tau protein levels in the blood of patients suspected of suffering from neurological disorders – an indication that was prevalent in the prior art as demonstrated by Grounds 1 and 2 below.

V. BACKGROUND

A. Summary of the '092 Patent

43. The Challenged Claims relate to a method for producing a patient bodily fluid sample having an analytically quantified amount of endogenous tau protein using an assay that is highly sensitive.[1] The '092 Patent acknowledges that tau was a known biomarker of "patients with neurodegenerative disease and head injuries." The concentration of tau in the cerebrospinal fluid (CSF) and blood "was known to increase with hypoxic events", however, because tau "must diffuse across the blood brain barrier" it may be present in the blood "in extremely low concentrations that are not reliably measurable by typical conventional immunoassays." Thus, while not expressly claimed – but how the examiner in original prosecution understood the claims – the inventors sought to use the more sensitive, well-known in the art, SMA to detect tau in the blood. EX-1001, 1:49-2:13.

[1] While not relevant to the obviousness challenges set forth herein, by its own terms the claimed "patient bodily fluid" is destroyed during the quantification step and, thus, there is no physical "product" that results from the claimed process.

44. A SMA interrogates proteins and other target analytes at the level of individual molecules. The '092 Patent does not purport to have invented SMAs or even a new SMA technique. Rather, as the '092 Patent acknowledges, SMAs were known in the art to provide ultra-high sensitivity and several different SMAs had been used to detect protein biomarkers in blood at extremely sensitive levels, including the *attomolar* range (*i.e.*, $1x10^{-18}$ moles/L levels). SMAs' ultra-high resolution was known to be useful for diagnosing disease, understanding the state of tissues, or finding treatments for disease. EX-1001, 16:27-17:41.

45. Instead, at a high level – which is the level at which the technique is claimed – the claimed method generally corresponds to three steps that are indicative of all prior art SMAs:

> Step (A) – obtaining a volume of blood, plasma or serum.
>
> This step is self-evident.
>
> Step (B) – diluting the volume of bodily fluid.
>
> Consistent with prior art SMAs, the concentration of the sample is diluted such that a single molecule of the target protein is isolated in an individual reaction vessel (as the name implies) for detection.

Step (C) – quantifying through the use of an assay a concentration of tau protein in the volume of bodily fluid, wherein the concentration of tau in the diluted sample is less than about 5 pg/ml, and a limit of quantification (LoQ) of the assay is less than about 0.2 pg/mL. Prior art SMAs were known to quantify blood protein levels at concentrations less than about 5 pg/ml, and using assays with LoQs that were <0.2 pg/mL and up to 10 times more sensitive than the levels claimed by '092 patent. And tau was a known biomarker for neurological disorders. As such, the claimed method amounts to nothing more than the obvious use of a known to be highly sensitive immunoassay, to quantify the concentration of a known biomarker, known to be present at low levels in blood. In short, all of the inventive work had already been performed by those who came before, rendering the Challenged Claims obvious.

B. Prosecution History

46. I understand from the prosecution history, the PO argued that following the publication of the invention, there was an alleged "sea-

change in the field" that "effectively created or contributed to creating the reasonable expectation of success that was absent at the time Applicant's invention was made…." Applicant argued that a 2021 review article by Ding (EX-1019) evidenced, "dramatic rise in publications with clinically relevant measurements of tau in blood", the "vast majority" of which "use Simoa® techniques described in the present Application." EX-1002, 493-610.

47. I also understand from the prosecution history that the PO did not explain how the alleged "rise" in tau-related publications was the result of both what is claimed and novel in the then pending, and now issued, claims (as opposed to being the result of prior art SMA techniques like the Quanterix Presentation or Duffy, PO's marketing of SMA technology, PO's public assertions of a dominating patent position, the publications reported in Ding being by PO or authors affiliated with PO, or improvements in tau antibody specificity; *see e.g.,* EX-1034 (PO's tau assay kit), EX-1039 (PO announcement of commercial availability of fully automated SiMoA platform), EX-1040 (key SiMoA platform patents press release), EX-1041 (key SiMoA platform patent press release); EX-1019 (Table 1, citations 27-29, 31, 35, 36, 39, 40, 41, 43, 47, 55, 56, 58, 59, 64 and 65 have authors affiliated with PO or '092 patent); EX-1042, at 67, 73

(citation 25 for "Simoa is a novel technology" is to "Epub ahead of print" concerning "The Simoa HD-1 Analyzer"). Nonetheless, the examiner allowed the claims, noting incorrectly in his Reasons for Allowance that "Ding table 1 lists Applicant's claimed assay (tau Simoa) used by at least forty-two (42) groups after the effective filing date of 4/12/11." EX-1002, EX-1002, 654-661 (Reasons for Allowance). I have reviewed Ding, and do not believe it represents an alleged "sea-change". In reality, Ding describes sixty-six studies (publications), not groups, wherein "ultrasensitive immunoassays" were used to measure tau in plasma. Ding further states that only 42 of these 66 studies used the Simoa assay. In regards to the "groups" statement, the authors on many of these Simoa publications were affiliated with the PO. And, in any event, as set forth below, the Challenged Claims are not expressly limited to SMA. EX-1019, 6-7.

C. Priority Date of the Challenged Claims

48. I understand the '092 Patent claims the benefit, through a series of continuation applications, to two provisional applications, the earliest of which was filed April 12, 2011. I have been instructed to assume that the earliest priority date is April 12, 2011.

D. Claim Construction

49. I have been informed to give each claim term in the Challenged Claims its plain and ordinary meaning in this proceeding. It is my opinion that no specific construction of any claim term is required because the prior art I have relied upon meets each of the claim terms under any reasonable construction.

E. Person of Ordinary Skill in the Art

50. With respect to the '092 Patent, a POSA in April 2011 would have been familiar with, and have a working knowledge of, molecular biology techniques including immunology techniques and assays, such as enzyme-linked immunosorbent assays (ELISA) and SMAs, and detecting tau in blood by immunoassays for diagnosing neurological conditions. A POSA would have knowledge of these concepts through a mixture of education, training and work experience such as by having a Bachelor's degree in molecular, cellular, or developmental biology, biochemistry, biomedical sciences, immunology, or neuroscience such as molecular neuroscience, or an equivalent degree, coupled with six years of laboratory experience; or a Master's degree in such fields with four years of laboratory experience; or a PhD in such fields with at least two years of laboratory experience.

F. State of the Art

51. This section describes the state of the art of molecular neuroscience that includes anti-tau antibodies, and assays that use these antibodies, especially for the detection or diagnosis of neurological diseases (including AD) as of April 2011. The prior art references, and the discussions of what was known to a POSA, are exemplary in nature, and relied on to provide factual support for my opinions, and can be considered by the Board to show the general state of the art, to identify motivation to combine the teachings of the primary references, to support reasonable expectation of success, to rebut any assertions of unpredictability in the art or unexpected results, and to rebut any assertions that the claims are directed patentable subject matter.

1. The Role of Tau in Alzheimer's Disease and Related Neurological Disorders

52. AD is a progressive neurodegenerative disorder associated with memory loss, spatial disorientation, and gradual deterioration of intellectual capacity. AD is estimated to effect more than six-million Americans (one in eight), and necessitates long-term care costing hundreds of billions annually. AD is characterized by the formation of abnormal clumps (amyloid plaques) and tangled bundles of fibers (neurofibrillary, or tau, tangles) in the brain. EX-1020.

53. Tau, a structural microtubule-associated protein, is mainly expressed in neurons and understood to have six isoforms. Neurons, or nerve cells, are responsible for sending and receiving messages between different parts of the brain, and from the brain to muscles and organs in the body. As illustrated, neurons are polarized cells with two types of cell extensions, dendrites and axons, whose establishment depends on a microtubule cytoskeleton.

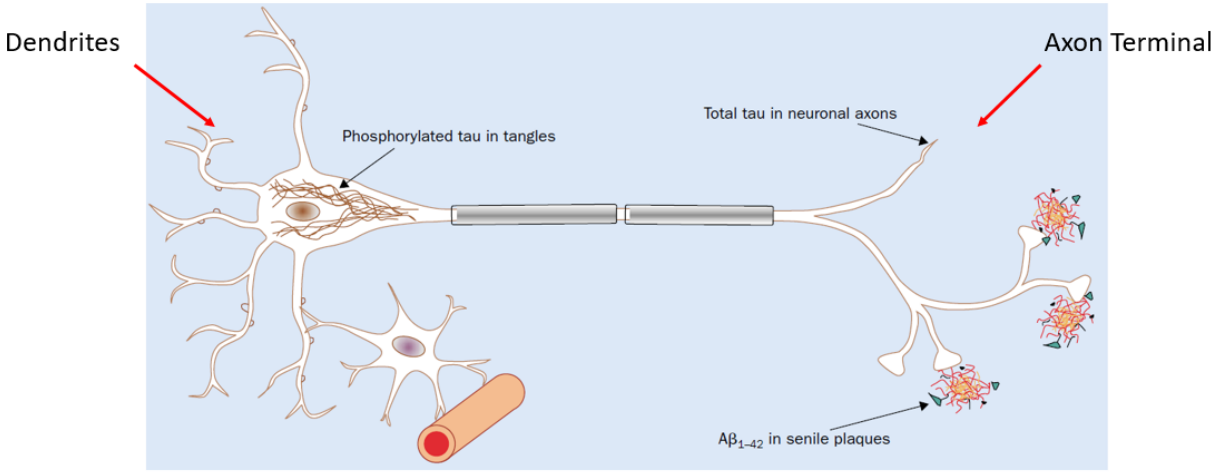


Figure 1. Schematic drawing of a neuron with an adjacent astrocyte and capillary. The central pathogenetic processes in AD and their corresponding biochemical markers are depicted. Total concentration of tau protein is a marker of neuronal and axonal degeneration, $A\beta_{1-42}$ concentration is a marker of plaque formation, and concentration of phosphorylated tau is a marker for hyperphosphorylation of tau and formation of tangles.

EX-1020; 515; EX-1032.

54. Normally, tau functions to promote the assembly of tubulin into microtubules, which helps stabilize their structure. These microtubules facilitate the transport of essential molecules within the neuron. EX-1021. However, in AD and other neurodegenerative diseases, tau becomes abnormally phosphorylated, causing it to detach from microtubules and

aggregate into tangled fibers called neurofibrillary tangles (NFTs). These NFTs disrupt the cell's internal transport system, impairing communication between neurons. Ultimately, plaques and tangles spread throughout the brain, the neurons stop functioning, and die. EX-1022, 9916. Key tau discoveries are illustrated below:

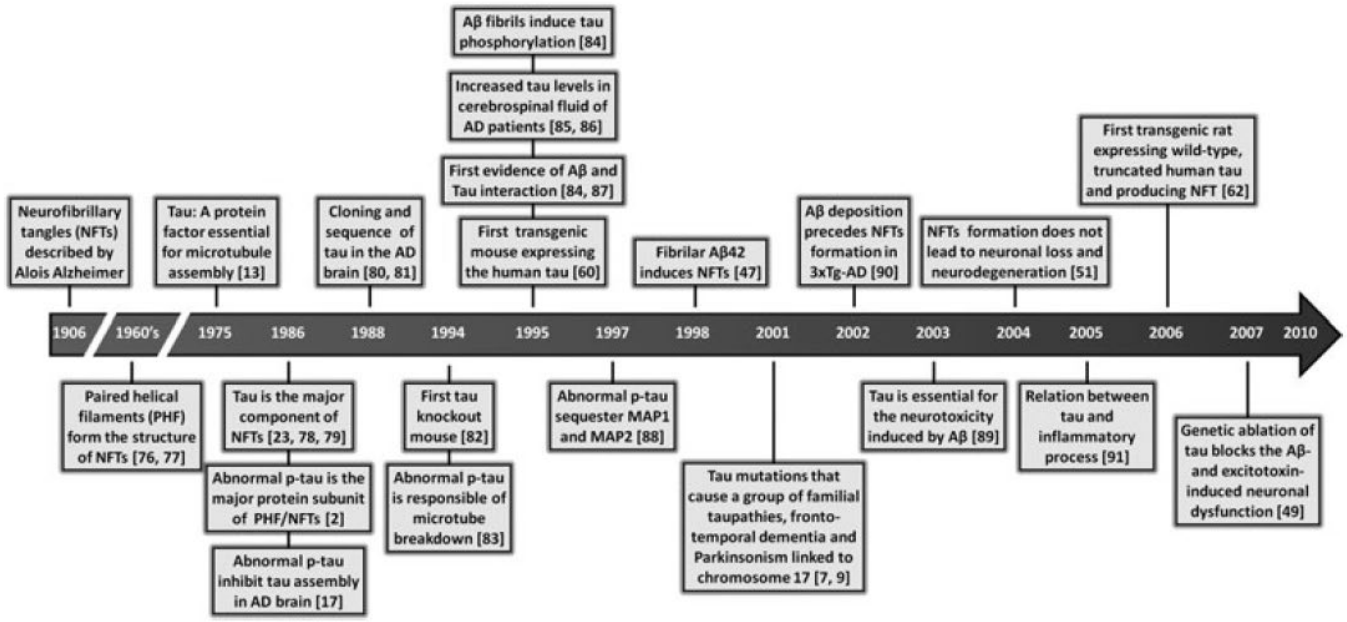


Figure 1 Key discoveries in tau protein research.

EX-1020, 514-515.

55. By the mid-1980's, tau became recognized as a biomarker – a substance indicative of disease – for early diagnosis and prognostic prediction of the changes in brain function and other cognitive disorders, including AD. Galasko describes a Working Group convened in 1998 by

the Alzheimer's Association and the National Institute on Aging to develop guidelines for identifying biomarkers for AD. A key recommendation was that biomarkers should be convenient, inexpensive and able to be measured sensitively and reliably and, thus, they logically suggested that blood would be a more convenient source of biomarker than CSF (which requires a complex and risky spinal tap). Moreover, because it was believed that AD pathology starts 20–30 years before its clinical onset, there was a recognized need to detect elevated tau levels in at risk patients as early as possible to prevent or slow disease onset. EX-1025, 342-43; EX-1029, 1424-25.

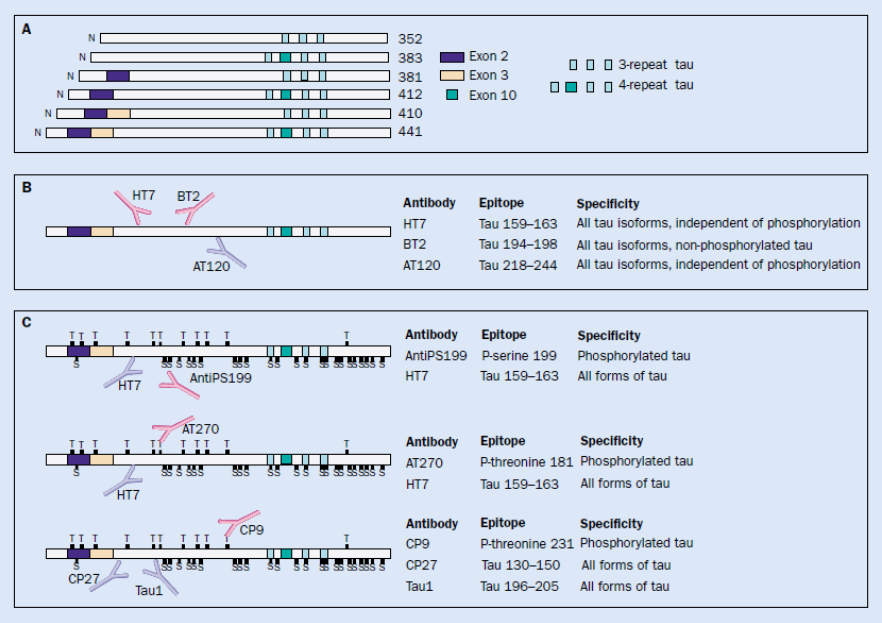
56. Thus, early studies explored tau as a biomarker in human CSF, blood, and blood components (*e.g.*, serum and plasma) to facilitate early diagnosis of neurological disorders in the brain. EX-1025, Abstract; EX-1008, 93-94, 122-140, 151-158, 163-167; EX-1017, 559; EX-1018; EX-1023, 1:20-29, 1:57-2:7, 2:33-38, 16:52-17:28; EX-1024 (immunoassay for tau); EX-1026.

57. Researchers focused on developing anti-tau antibodies. These antibodies were first developed as polyclonal antibodies, with monoclonal antibodies that detect all isoforms of tau (*i.e.*, "total tau" or "t-tau"), independent of phosphorylation, developed thereafter. For example, in

1993, Vandermeeren taught a sandwich ELISA using a monoclonal antibody directed to human tau in CSF. With this technique, the detection limit for tau was less than 5 pg/ml of CSF. EX-1026, Abstract, 1832-33.

58. In 2000, Hulstaert explored a method for the early detection and quantification of CNS damage by monitoring tau levels in either CSF or blood. Hulstaert explains that tau was a known indicator of CNS damage caused by anoxia or ischemia, including during stroke, cerebral infarction, thrombosis, cerebral hemorrhage, and CO poisoning, or caused by chemical or physical agents, or a combination of those mechanisms. EX-1027, cls 1, 2, 4; EX-1028, Examples I-V.

59. By 2003 phosphorylated tau ("p-tau") was recognized as a strong candidate biomarker for AD, with total tau being a general biomarker for neuronal damage, and with the existence of numerous antibodies against t-tau, and various phosphorylated forms of tau:

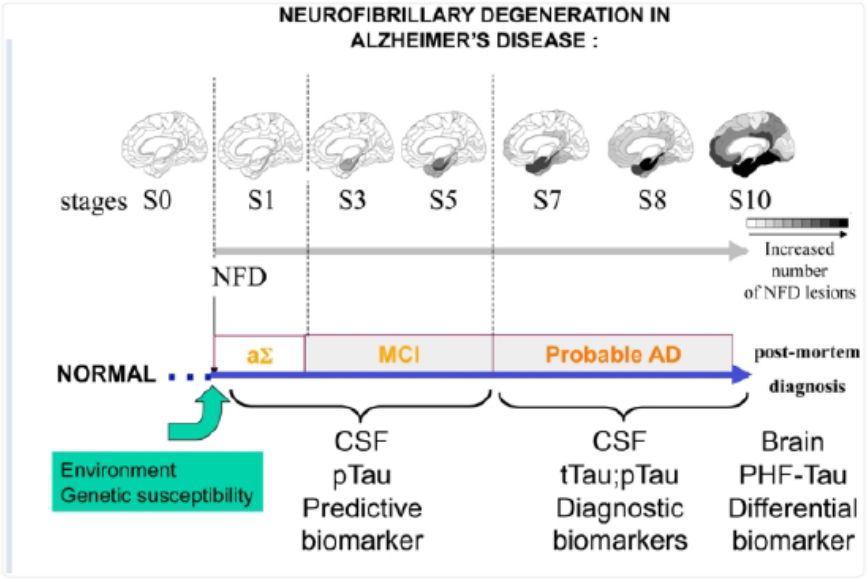


EX-1032, 606, 607.

60. In 2006, Wunderlich reported elevated tau (including phosphorylated tau) in serum of acute ischemic stroke patients, and experiments that detected 30 pg/ml of tau in blood at 3, 6 and 12 hours after stroke, concluding that tau is a useful predictor of outcome in stroke patients. EX-1017, 559, 563 and Fig. 1b. Mortberg also reported that the presence of tau in blood or CSF is a marker for axonal loss of grey matter axons, suggesting a prognostic value for tau in stroke, closed head injury, mild traumatic brain injury, and bilirubin-induced neurotoxicity. These uses of tau are very important and expand the use of tau to a number of "neurological conditions" in addition to AD. EX-1018, 19-20. Researchers recognized that TBI may lead to an increased risk of

developing neurological disorders, including AD, Parkinson's disease and other disorders. EX-1037; EX-1038. As Galasko explained, because "tau can be released from damaged neurons or axons regardless of the cause, any disorder that damages or destroys enough neurons will release tau into CSF." EX-1025, 342.

61. By 2008, it was well known to use assays for p-tau and t-tau during the course of AD, including because as between p-tau181 and p-tau231 – the most studied phospho-epitopes of tau – p-tau181 occurs later in AD than p-tau231:



EX-1031, 364.

62. Thus, by 2008, there were selective, sensitive antibodies that could well discriminate among the forms of tau proteins – including so that one could discriminate AD from normal aging – allowing the POSA to

readily run a more sensitive assay, such as SMA, with an expectation of success. EX-1031, 373. Indeed, as the admitted in the specification of the '092 patent, the "six known natural-occurring tau proteins" are "well-known in the art", including "phosphorylated" tau and capture antibodies with affinity for tau (i.e., anti-tau antibodies) were both well-known and commercially available in the art. EX-1001, 8:37-51; 33:34-40.

2. SMA and Detecting Tau

63. Rapid and reproducible methods that implement high-sensitivity and low-level analyte detection are the cornerstone of analytical chemistry. Scientists have been focused on improving the sensitivity and detection limit of such methods for decades. EX-1016, 6286-6287.

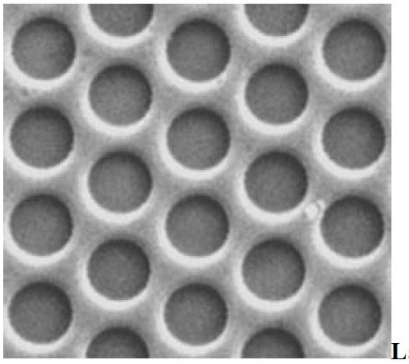
64. Early immunoassay techniques for quantifying low-level analyte in a sample employed amplification procedures designed to increase the number of reporter molecules producing a signal. These processes included, *inter alia*, ELISA for amplifying the signal in antibody-based assays. While these methods allowed low-level analyte detection, they often involved complex procedures and required a large number of molecules for the aggregate signal to rise above the detection threshold. These disadvantages limited the sensitivity and dynamic range (*i.e.,* the range of concentrations that can be detected) of these techniques.

Moreover, the amplification procedures often lead to non-specific binding (*i.e.*, binding of analyte or reporter species non-specifically to sites other than those expected). This, in turn, amplified the background signal as well as the true signal resulting in non-improved sensitivity. EX-1016, 6286.

65. By the early 2000's, scientists developed new techniques for quantifying low-level target analytes. Rather than amplify the target analyte in a sample – which caused the above disadvantages – these techniques focused on digitizing the signal generated from immunoassays and measuring single molecules. Aided by the development of nanotechnologies (including optical fiber bundles and flow technologies), scientists began using a variety of techniques to separate true signal from background signal (including densely-packed arrayed structures with micrometer-sized elements), aimed at capturing and detecting *individual molecules* of a target analyte, as discussed in more detail below.

66. Bourzac describes one of these SMAs. A single optical fiber bundle is made up of thousands of individual glass threads. As illustrated by Bourzac below, by dipping optical fibers in acid, the individual threads can be used to etch a substrate with tens of thousands of microwells, one at the tip of each thread, effectively creating a large array of nanoscale test

tubes. Each microwell can then be coated with protein-capturing antibodies. Using these femtoliter-sized reaction chambers (*i.e.,* one-quadrillionth or 1 x 10^{-15} of a liter), the reactor volumes are small enough such that they contain either zero or one analyte molecule. The individual optical threads in contact with each well can be used to carry both excitation and emission light to and from the wells, thereby enabling remote interrogation of the well contents. EX-1011, 1.



Lab on a tip: Wells carved into the tip of an optical fiber allow researchers to detect single proteins in blood samples. Each well in this image is about 2.5 micrometers in diameter and sits at the tip of an individual thread of an optical fiber.

67. As excitation light is shone on the microwell, the sandwiched antibody and enzyme undergo a reaction that produces fluorescent light. The light travels back up the optical fiber, without signal "cross-talk" between fibers. EX-1011, 1.

68. The presence or absence of a fluorescent product in each reaction vessel provides a binary readout ("on" or "off"). At very low target concentrations, the ratio of the number of analyte molecules to the number of wells assumes a "Poisson distribution", where either a single

analyte molecule or no analyte binds to each well. The percentage of reaction vessels occupied by enzyme molecules can then be correlated to the bulk concentration by simply counting the number of "on" or "off" reaction vessels. Whereas the detection limits of ELISA was between 1-10 pg/ml of protein in blood (*e.g.*, 10^{-10} to 10^{-12} g/ml), SMA (sometimes called "Digital ELISA") allowed attomolar level detection – many thousand times more sensitive (*e.g.*, 10^{-16} to 10^{-18} g/ml). EX-1009, 522.

69. By the mid-2000's, SMAs were well described and recognized as a simple, low-cost approach to single molecule detection that was inherently scalable and had the ability to detect numerous different analytes (including proteins and small molecules). Scientists had developed several different single-molecule assay techniques. In 2007, my colleagues and I assessed a SMA technology called Erenna Immunoassay System ("EIS") based upon flow technology to ascertain the limit of detection (LoD) and the dynamic reporting range of a series of ten different biomarkers in blood plasma, and achieved a LoD of 0.01 pg/mL. I expressed the prevailing view of the "utmost importance" of understanding its potential applications for detecting protein biomarkers for disease diagnosis, monitoring, and management. EX-1012, 1993-94.

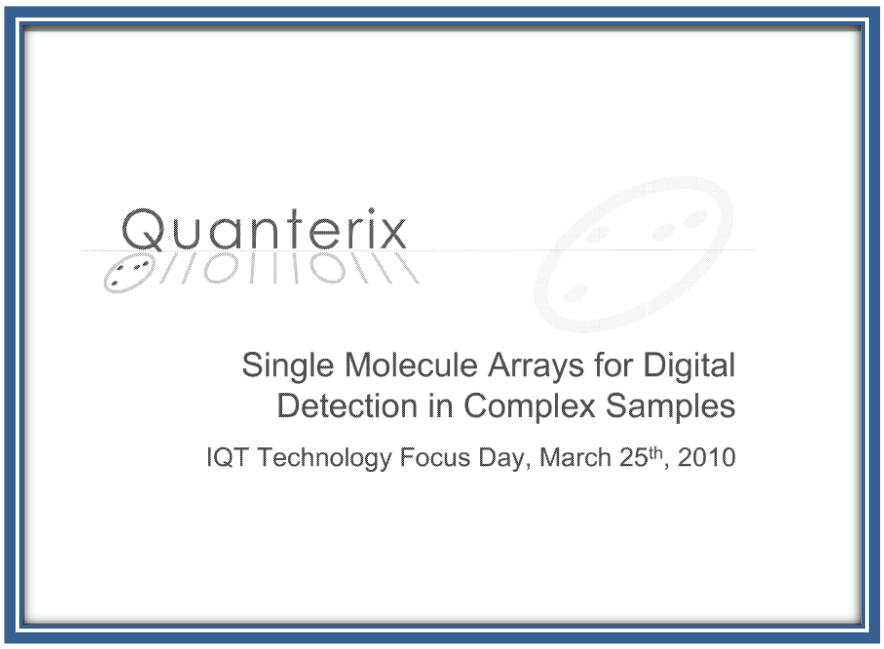
70. Scientists at Tufts University were also developing SMAs for detection of disease, neurological conditions and cancer. Dr. David Walt studied β-galactosidase using a 1 mm diameter fiber optic bundle with 24,000 individually sealed, femtoliter microwell reactors. Walt applied a Poisson statistical analysis to obtain the concentration of single molecules of β-galactosidase. In particular, Walt noted that this approach would be "useful for single molecule enzymology and ultrasensitive bioassays" and concluded that the "future of this technology will permit studies with multiple different enzymes and will push the limits of ultralow detection for protein and DNA targets using enzyme-linked detection methods in conjunction with the high-density femtoliter array." EX-1009, Abstract, 523; EX-1011.

71. My work and that of Walt was built upon by Duffy. Duffy taught a method of detecting protein biomarkers in body fluid samples such as "blood, serum, plasma, urine, saliva, tissue, organ, or the like", using femtoliter arrays to analyze single molecules for detection of disease, neurological conditions and cancer. In order to increase the dynamic range of the assay, Duffy taught to dilute the sample by a factor of 10 or more. EX-1015, 144, 213.

72. The diluted sample was exposed to a substrate with a plurality of capture components (antibodies) immobilized thereon. As analyte molecules associated with the capture components, they bound together to form capture complexes (*i.e.,* antibody-antigen complexes) immobilized on the substrate surface. At least a portion of the capture complex was then dissociated from the substrate, and analyzed using a femtoliter array "such that at least some… reaction vessels contain zero particle-containing complexes and at least some… reaction vessels contain only one or at least one particle-containing complexes." EX-1015, 224-270.

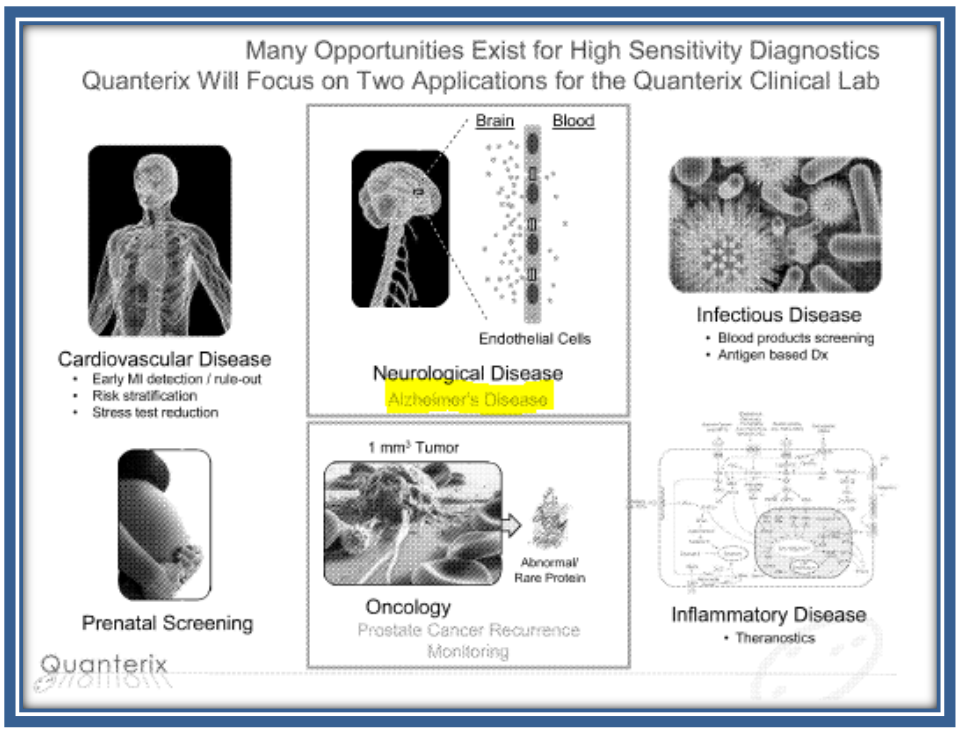
73. The presence and amount of disassociated species can then be detected and quantified, and the analyte concentration is determined by a Poisson distribution analysis of the number or fraction of reaction vessels that contain a dissociated species. Like other single molecule techniques, Duffy's methods were capable of detecting ultra-sensitive levels of analyte at about 50×10^{-15}. EX-1015, 10, 185, 198.

74. On March 25, 2010, Duffy made a presentation on behalf of the Petitioner titled "Single Molecule Arrays for Digital Detection in Complex Samples" presented at the IQT Technology Focus Day ("the Quanterix Presentation") (EX-1033).



EX-1033, slide 1, EX-1002, 493-610.

75. The Quanterix Presentation teaches that SMA is capable of detecting ultra-sensitive levels of proteins (*e.g.*, TNF-α and IL-6 at 0.20 fM, and PSA at 0.10 fM), small molecules (below 10 fM), as well as single cell arrays to detect RNA and DNA, and discusses several "current applications" of SMA, including neurological diseases, such as AD:



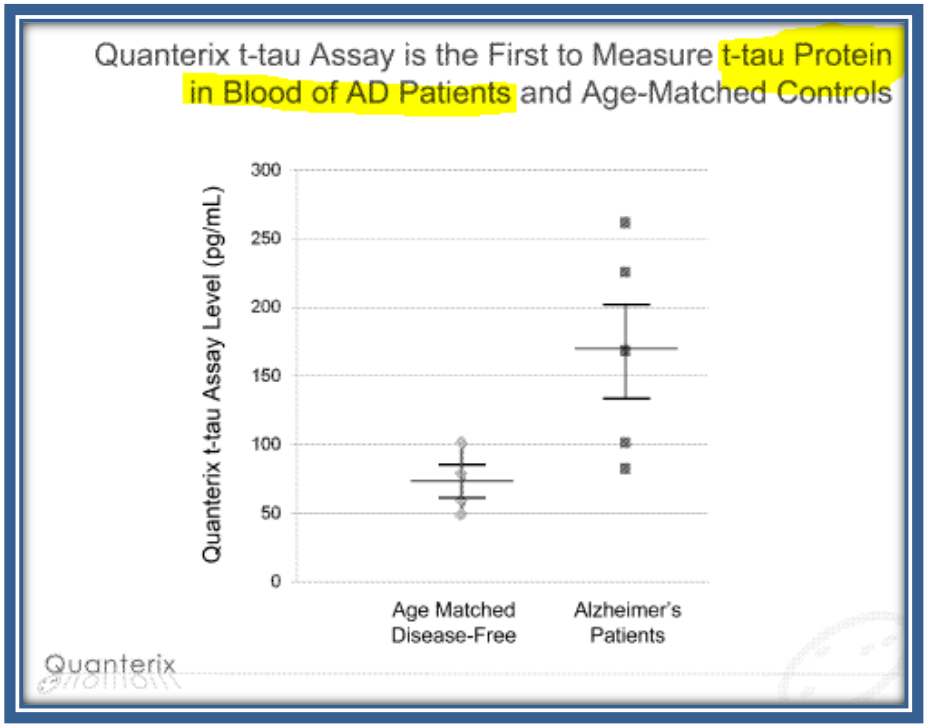
EX-1033, slides 9, 11, 15-17, 19.

76. In particular, the Quanterix Presentation describes the use of SMA to detect tau in the blood of patients suffering from AD and other TBI, quoting Dr. Rudolf Tanzi, a neurologist at Massachusetts General Hospital, who explains that an ideal "serum marker for TDI" is "Tau":

> You want a nice serum marker for TBI, you do it through Tau. That is where you may have a use for Tau because the blood brain barrier is damaged. Then you may be able to get some assessment of the degree of injury following a TBI by looking at Tau in the plasma.

EX-1033, slide 29.

77. The Quanterix Presentation reports the results of a "Quanterix t-tau Assay", based upon SMA technology, that measured t-tau in blood of AD patients and age-matched controls:



EX-1033, slide 22.

78. The Quanterix Presentation notes that SMA provides an "[a]lternative non-invasive source for identification of low abundance neuronal specific proteins" as compared to CSF tests. It further notes that "[a] rapid blood-based test for TBI would have significant value" in view of its ability to provide "real-time assessment of new/worsening neurologic injury", to "predict secondary pathologies (*e.g.*, elevated JCP, ischemia)",

and "detect[] mild injury that cannot be observed with CT/MRI." EX-1033, slide 29.

79. Duffy's methods were soon put to diagnostic use. Quanterix began developing an "Alzheimer's Disease test panel" and published a press release announcing its ability to use SMA technology to detect the biomarkers for Alzheimer's "with a simple blood test, even though they are present in the blood at exceedingly low concentrations, well below the limit of detection achieved by standard testing methods." Quanterix explained the benefits of its test, including "eliminating the need for invasive medical procedures, shortening time to diagnosis, and reducing the use of expensive imaging modalities." Quanterix also taught other useful applications, including as diagnostic test for "other neurological disorders, including Parkinson's disease, bipolar disorder, stroke and traumatic brain injury (TBI)." EX-1030. At the same time, other competitors in the field, including my company Singulex Inc., were already offering commercially available SMA directed at testing biomarkers for AD. EX-1036.

80. Accordingly, long before the '092 patent earliest filing date, the state of the art was using SMA for calculating concentrations of tau in blood as a biomarker for numerous neurological conditions, including AD.

VI. IDENTIFICATION OF HOW THE CHALLENGED CLAIMS ARE UNPATENTABLE

A. Ground I: Rissin in view of Todd

1. The Rissin Reference

81. Like the '092 patent, Rissin teaches performing a SMA assay (digital ELISA) to measure low concentrations of protein biomarkers in blood and blood serum for clinical diagnosis of healthy and disease states and to monitor disease progression, "us[ing] of arrays of femtoliter-sized reaction chambers" that "can isolate and detect single enzyme molecules":

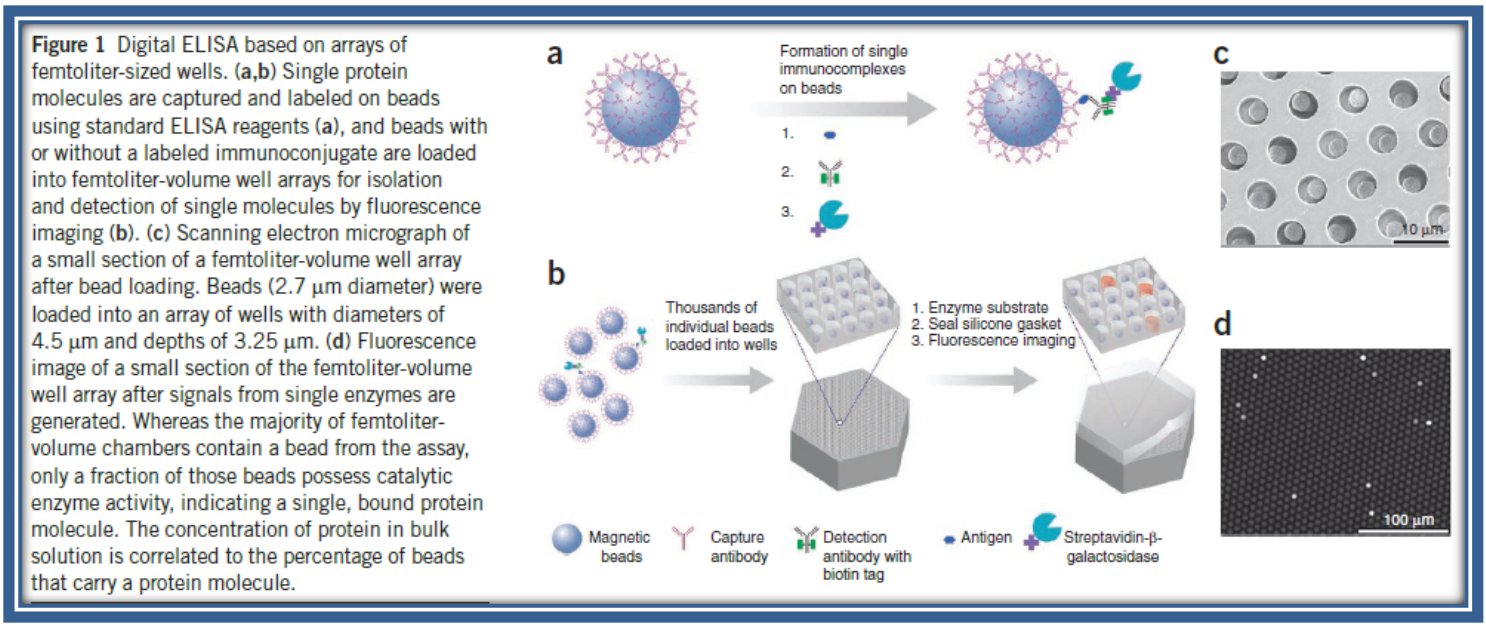


Figure 1 Digital ELISA based on arrays of femtoliter-sized wells. (**a**,**b**) Single protein molecules are captured and labeled on beads using standard ELISA reagents (**a**), and beads with or without a labeled immunoconjugate are loaded into femtoliter-volume well arrays for isolation and detection of single molecules by fluorescence imaging (**b**). (**c**) Scanning electron micrograph of a small section of a femtoliter-volume well array after bead loading. Beads (2.7 µm diameter) were loaded into an array of wells with diameters of 4.5 µm and depths of 3.25 µm. (**d**) Fluorescence image of a small section of the femtoliter-volume well array after signals from single enzymes are generated. Whereas the majority of femtoliter-volume chambers contain a bead from the assay, only a fraction of those beads possess catalytic enzyme activity, indicating a single, bound protein molecule. The concentration of protein in bulk solution is correlated to the percentage of beads that carry a protein molecule.

EX-1005, Fig. 1.

82. Rissin describes disadvantages with conventional analyte detection techniques, including measuring "proteins at concentrations above 10^{-12} M", whereas "serum concentrations of the majority of proteins

important in… neurological disorders", "rang[e] from 10^{-16} to 10^{-12} M." Prior art analyte detection techniques were not thought to reliably measure protein biomarker concentrations. EX-1005, 598.

83. Rissin teaches that SMA solves these problems. "As [SMAs] provide superior label sensitivity over conventional assays, substantially less detection antibody (~1 nM) and enzyme conjugate (1−50 pM) are needed to detect binding events as compared with conventional assays…. The decreased label concentration reduces nonspecific binding to the capture surface, resulting in much lower background signals." EX-1005, 598.

84. Rissin acknowledges that others were developing ultra-sensitive and single molecule assays. For example, Rissin discusses that Mirkin "used labels based on gold nanoparticles and DNA biobarcodes to push the detection of proteins into the low femtomolar range; and… detect[] 10 fM of PSA in serum." Rissin also discusses Todd, who "developed flow-based methods for serially detecting single fluorescently labeled detection antibodies released from immunocomplexes formed on solid substrates." Rissin sought to "exploit the ability of [SMAs] to trap and detect single enzymes to detect single enzyme–labeled proteins" in order to further improve upon these ultra-sensitive technologies "using the

same reagents as the gold standard for detecting proteins, namely, the ELISA." EX-1005 at 595.

85. According to Rissin: "In the first step… (Fig. 1a), a sandwich antibody complex is formed on microscopic beads (2.7 μm diameter), and the bound complexes are labeled with an enzyme, as in a conventional bead-based ELISA." "When assaying samples containing extremely low concentrations of protein, the ratio of protein molecules (and the resulting enzyme-labeled complex) to beads is small (typically <1:1) and, as such, the percentage of beads that contain a labeled immunocomplex follows a Poisson distribution." At low protein concentrations, "the Poisson distribution indicates that beads carry either a single immunocomplex or none." "At ratios of enzyme to beads greater than ~1:10, the fraction of active beads becomes much higher, and Poisson statistics show that there are a significant number of beads with multiple enzymes." Thus, to ensure protein concentrations in the sample are sufficiently low, a "fourfold dilution factor is typically used to reduce matrix effects in immunoassays." EX-1005, 595-98.

86. Rissin explains SMAs' operation, including its ability to "confin[e] the fluorophores generated by individual enzymes to extremely small volumes (~50 fl), ensuring a high local concentration of fluorescent

product molecules." To achieve this confinement, "beads are loaded into an array of femtoliter-sized wells." A "droplet of fluorogenic enzyme substrate", is added causing those beads "possessing a single enzyme–labeled immunocomplex" to fluoresce. "By acquiring time-lapsed fluorescence images of the array… it is possible to distinguish beads associated with a single enzyme molecule ("on" well) from those not associated with an enzyme ("off" well)." EX-1005, 596, Fig. 1; "[P]rotein concentration in the test sample is determined by counting the number of wells containing both a bead and fluorescent product relative to the total number of wells containing beads (Fig. 1d)."

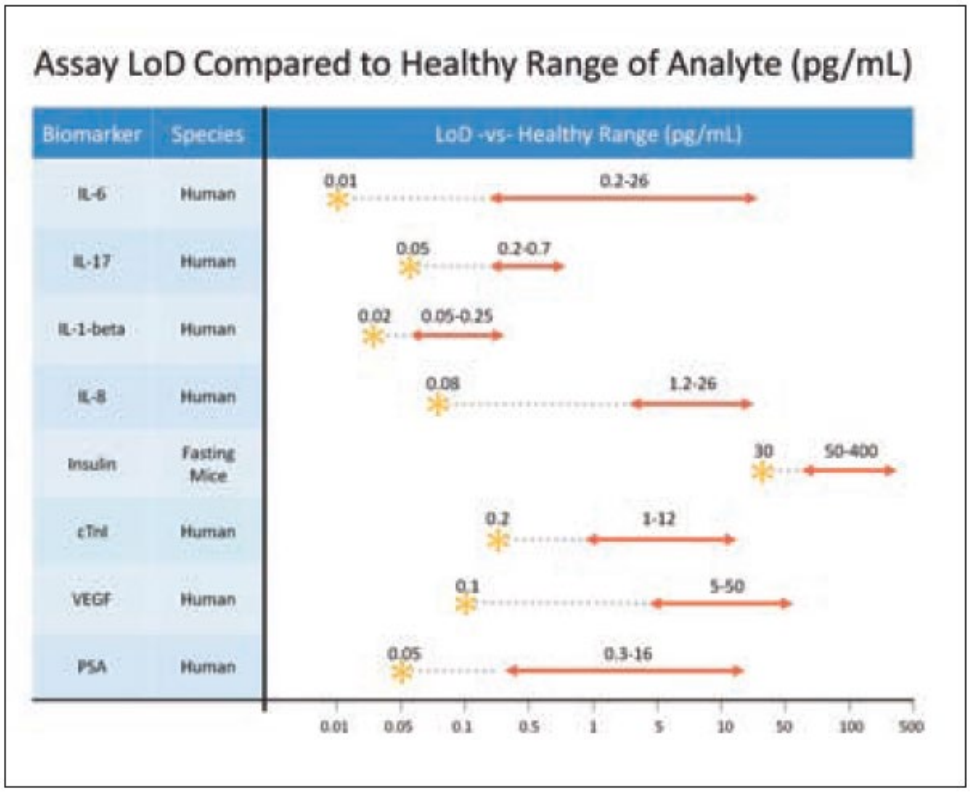
87. "As [SMAs] enable concentration to be determined digitally rather than by using the total analog signal," Rissin dubbed his approach "digital ELISA." The lowest concentration of enzyme conjugate detected by Rissin was 350 zeptomolar (zM) and the calculated LOD was 220 zM, corresponding to an increase in sensitivity over prior art ELISA "of a factor of about 68,000." EX-1005, 596.

2. The Todd Reference

88. Todd describes the use of a SMA technology developed by Singulex, Inc. (commercially sold as the "Erenna Immunoassay System" or "EIS") that could be used to detect tau biomarker in the blood plasma of

patients for early detection of AD. Like the claimed invention, Singulex's SMA utilizes "paramagnetic microparticles (MPs) as the solid phase for immune-capture and detection of analyte from a complex biological sample, providing enhanced specificity, sensitivity and precision" at "sub-picogram levels." Todd demonstrates that its SMA is capable of measuring numerous protein biomarker analytes in human blood plasma with a LoD of at least 0.01 pg/ml. EX-1006, 53.

Figure 4
Erenna immunoassay system LoD compared to the measured range of analyte quantified in healthy human subjects



EX-1006, Fig. 4.

89. Todd describes the challenges historically faced by translational scientists when detecting and defining the onset of disease

using protein biomarkers. While protein biomarkers provide a standardized metric for evaluating disease states, their application is often limited by the sensitivity of assay used for their detection. For example, "assay sensitivity is often what determines the lower limit of the threshold and the diagnostic cut-off value" for disease onset. "The threshold system only reveals the tip of the iceberg when it comes to disease states." Often, information that "resides beneath the 99th percentile threshold value for a clinically relevant biomarker… could be used to diagnose, stratify, determine risk, or aid in disease prevention." In addition, because the development of a disease state is a continuum rather than "an on/off proposition", disease onset and diagnosis is "difficult to mark" and "a common cause of diagnostic failures." EX-1006, 51-53.

90. Todd specifically highlights AD as "an excellent example" of a disease state having a "prolonged disease onset leading towards diagnostic failure." Todd explains that, because "the only definitive diagnosis of AD is a post-mortem examination of brain tissue… clinical diagnosis of this disease is diagnosed by ruling out other probable causes for the symptoms of AD, which are usually only apparent after significant disease progression has occurred." As a result, "there is no reliable way at this time to clinically define when this complex disease officially starts, and an

FDA approved molecular diagnostic for AD is desperately needed." EX-1006, 53.

91. In this way, Todd explains that "protein biomarker programmes in AD and other disease areas can benefit immensely from the application of novel immunoassay technologies with ultra-sensitive detection" such as SMA. Todd emphasizes that "[t]he need for an early diagnosis for AD is especially important as new therapies for AD are developed, which provide better patient outcomes when administered early in the course of the disease." Todd teaches that the "solution" is "integrating novel technology into next generation immunoassay systems" such as SMA, and specifically identifies "tau proteins" as one of two known protein biomarker candidates for clinical diagnosis of AD. EX-1006, 52-53.

3. The Motivation to Combine the Teachings of Rissin in view of Todd

92. As discussed in the State of the Art Section, the use of SMA to detect ultra-low concentrations of tau biomarker in the blood of patients suspected of having a neurological disorder was already known in the art. EX-1033, slides 9, 11, 15-17, 19; A POSA would have been motivated to use the SMA procedure of Rissin to quantify endogenous tau in the blood of patients suspected of having a neurological disorder as taught by Todd

to arrive at the inventions claimed by the Challenged Claims for several reasons.

93. Rissin and Todd, being in the same field of endeavor as the '092 patent, are analogous art. Both teach SMAs to quantify ultra-low concentrations of analytes in patients' blood for detecting and diagnosing neurological diseases. Indeed, Rissin expressly cites to the work of Todd 2007 (EX-1012) as "a promising approach for measuring extremely low concentrations of proteins" using "the isolation and detection of single protein molecules." Rissin notes that Todd 2007 has "developed flow-based methods for serially detecting single fluorescently labeled detection antibodies that have been released from immunocomplexes formed on solid substrates." EX-1005, 595;

94. Both Todd and Rissin speak to measuring tau blood and serum concentrations for the detection and diagnosis of AD. Specifically, Todd teaches that the use of SMAs provide a "solution" that would enable the ultra-sensitive detection of tau as a biomarker for the detection and diagnosis of AD. Todd explains the numerous benefits provided by the ultra-low diagnostic sensitivity and strategic applications of SMAs, including allowing for earlier detection of disease, more defined disease diagnosis criteria, and improving data relevant to critical new drug

development decisions. Todd explains that the "ultimate diagnostic challenge is to detect the onset of disease as early as possible" which "correlate[s] with better patient outcomes." By enabling highly sensitive biomarker detection, including detection of tau protein biomarkers, SMA technology is able to establish baseline levels in healthy subjects, track therapeutic effects of the new therapy, aid in the early detection of adverse events, and provide data to support preclinical efficacy and safety studies which enhances drug development. EX-1006, 56.

95. Rissin, too, recognized the application of SMAs to the detection and diagnosis of AD. Specifically, Rissin notes that "serum concentrations of the majority of proteins important in… neurological disorders… are thought to range from 10^{-16} to 10^{-12} M" and, in support, cites to two articles, EX-1025, EX-1029, discussing tau protein as a blood biomarker for AD. Rissin recognized the improved "diagnostic benefits" of SMAs, including their ability to detect and establish diagnostic threshold values for key biomarkers using a LoD that far exceeds the highest sensitivity of prior-art assays, presents a clear application for SMAs. Rissin, thus, directs the POSA to use Rissin's SMA technology to quantify ultra-low concentrations of tau in blood as a biomarker for neurological disorders such as AD. EX-1005, 595.

96. A POSA understood that while the SMAs of both Rissin and Todd were highly sensitive and had known diagnostic benefits, Rissin's SMA provided a more streamlined process that reduced the number of steps while still "detect[ing] proteins in serum at subfemtomolar concentrations". While both SMAs utilize antibodies disposed on a plurality of capture objects (*i.e.*, beads) to form capture complexes with analyte molecules, the Todd SMA includes the additional steps of releasing a portion of the capture complexes, and determining the concentration of the analyte using a portion of the released complexes. EX-1005, 595-599; EX-1006, 53; EX-1012 (describing the Erenna Immunoassay system developed by Singulex and discussed in Todd); In contrast, building on the earlier SMA technologies, Rissin sought to "simplify[] the logistics of the assay", including by eliminating the additional release steps present in the Todd SMA. EX-1005, 598-599.

97. A POSA recognized that the additional release steps taught for the Todd SMA were cumbersome, more complex, more time consuming and required additional reagents compared to Rissin. Moreover, a POSA recognized that the additional steps of Todd increased expense and further introduced additional and unnecessary opportunities for user error. Thus, a POSA recognized the benefit provided by the teaching of Rissin which

eliminated these additional release steps and their associated complexity, cost, and error.

98. Moreover, a POSA would have been confident that using Rissin's simplified SMA to quantify tau would have been successful because it was simply using a known tau antibody with a known technology (a simplified SMA) for its intended purpose: quantifying low-concentration protein analytes. Indeed, Rissin demonstrates its success at quantifying low-concentration protein analytes using two of the same blood proteins that were studied in Todd, *i.e.,* PSA and TNF-α. Rissin reported a similar LoD for these proteins as Todd, which a POSA understood were far more sensitive than the detection limits claimed in the Challenged Claims. *Compare* EX-1006, 56 (disclosing LoD of 0.20 pg/ml for TNF-α, and 0.05 pg/ml for PSA) *with* EX-1005, 597-98 (disclosing LoD of 2.5 fg/ml for TNF-α, and 1.5 fg/ml for PSA).

4. Detailed Application of Rissin in view of Todd

a. <u>Claim 1</u>

i. A method of producing a bodily fluid sample of a patient suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein, said method comprising:

99. To the extent the preamble is a limitation, Rissin discloses a method for performing simplified SMA to quantify blood protein concentrations for clinical diagnosis of neurological disease, citing two articles, EX-1025, EX-1029, discussing tau protein as a blood biomarker for AD. EX-1005, 595. Moreover, Todd teaches performing SMA for the detection and diagnosis of AD and expressly identifies tau proteins as one of two "hopeful candidates" to serve as an FDA approved biomarker for clinical diagnosis of AD. EX-1006, 53. A POSA would have been motivated to modify the teachings Rissin with the teachings of Todd in order to quantify concentrations of tau protein in blood for clinical diagnosis of neurological disease for the reasons discussed in the Motivation to Combine Section. This modification results in a *method of producing a bodily fluid sample of a patient suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein*, consistent with the discussion in the State of the Art section demonstrating that a POSA understood tau was a known indicator of TBI from a hypoxic or ischemic event, including cardiac arrest, stroke or thrombosis. EX-1027, cls 1, 2, 4; EX-1018 at 19-20.

ii. (A) obtaining a volume of bodily fluid comprising blood, or a blood component selected from plasma and serum,

100. A POSA reading Rissin understood that it includes the step of obtaining a volume of body fluid. Rissin teaches "detecting very low concentrations of proteins in blood using digital ELISA" including by measuring "serum samples from patients who had undergone radical prostatectomy" [*a volume of bodily fluid comprising blood, or a blood component selected from plasma and serum*]. EX-1011, 1-2. A POSA understood that, in order to perform such a blood-based assay, a sample of blood must first be "obtained."

iii. (B) diluting the volume of bodily fluid, and

101. Rissin discloses this limitation. Rissin discloses that the whole-serum samples collected for use in the SMA arrays "were diluted 1:4 in buffer and measured using the digital ELISA" [*diluting the volume of bodily fluid*]**.** EX-1005, 598; Fig. 3a (teaching "25% serum"). Rissin teaches that a "fourfold dilution factor is typically used to reduce matrix effects in immunoassays" and so that the resulting enzyme-labeled complex will "follow[] a Poisson distribution" wherein "beads carry either a single immunocomplex or none." Dilution is an established important method used by those skilled in the art. EX-1005, 597; 598; Fig. 3 (showing "final concentration of spiked protein in the *diluted* sample"). This, in turn, allows for single-molecule counting of labeled protein

molecules such that the protein concentration in the original test sample can be determined. EX-1005, 596.

iv. (C) quantifying through the use of an analytical protein concentration measurement assay a concentration of tau protein in the volume of bodily fluid to produce the bodily fluid sample containing the analytically quantified amount of endogenous tau protein;

102. Rissin in view of Todd discloses this limitation.

Rissin makes "use of arrays of femtoliter-sized reaction chambers" called "single-molecule arrays" (SMAs) that "can isolate and detect single enzyme molecules" [*quantifying through the use of an analytical protein concentration measurement assay*]. EX-1005, Fig. 1; Specifically, Rissin's Fig. 1 illustrates Rissin's *analytical protein concentration measurement assay*:

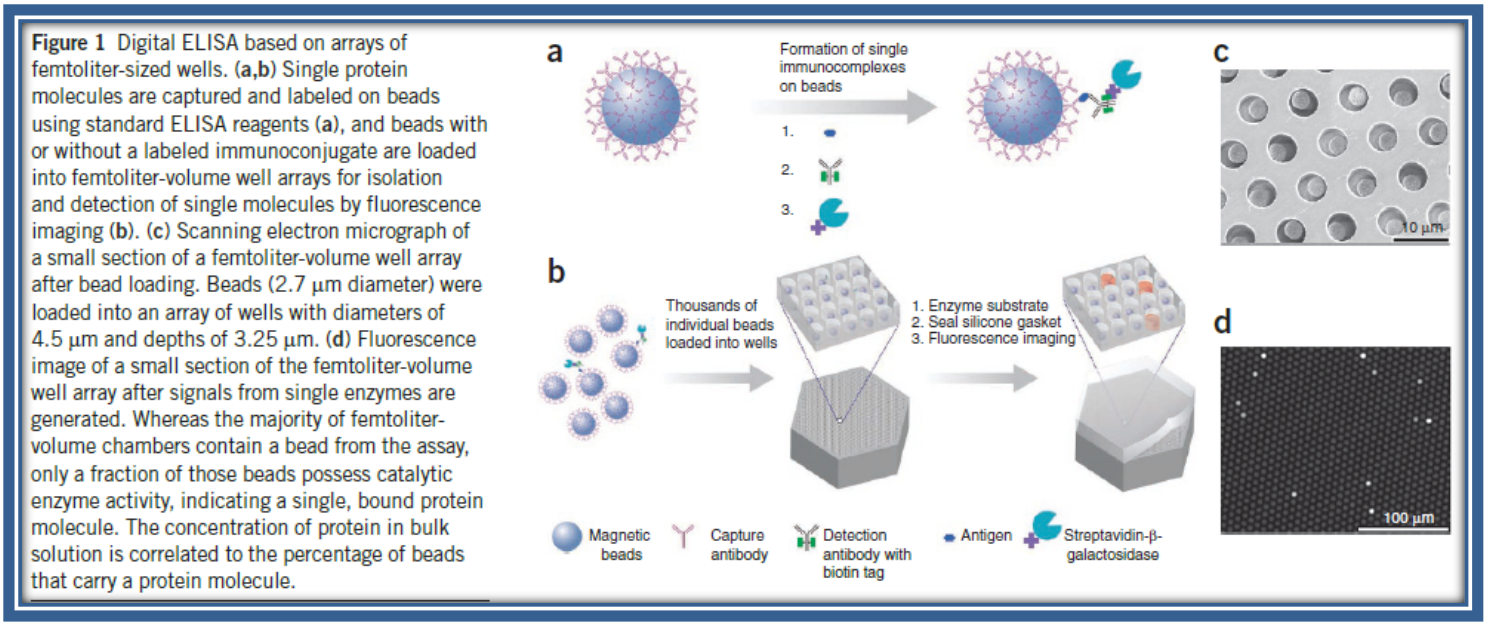
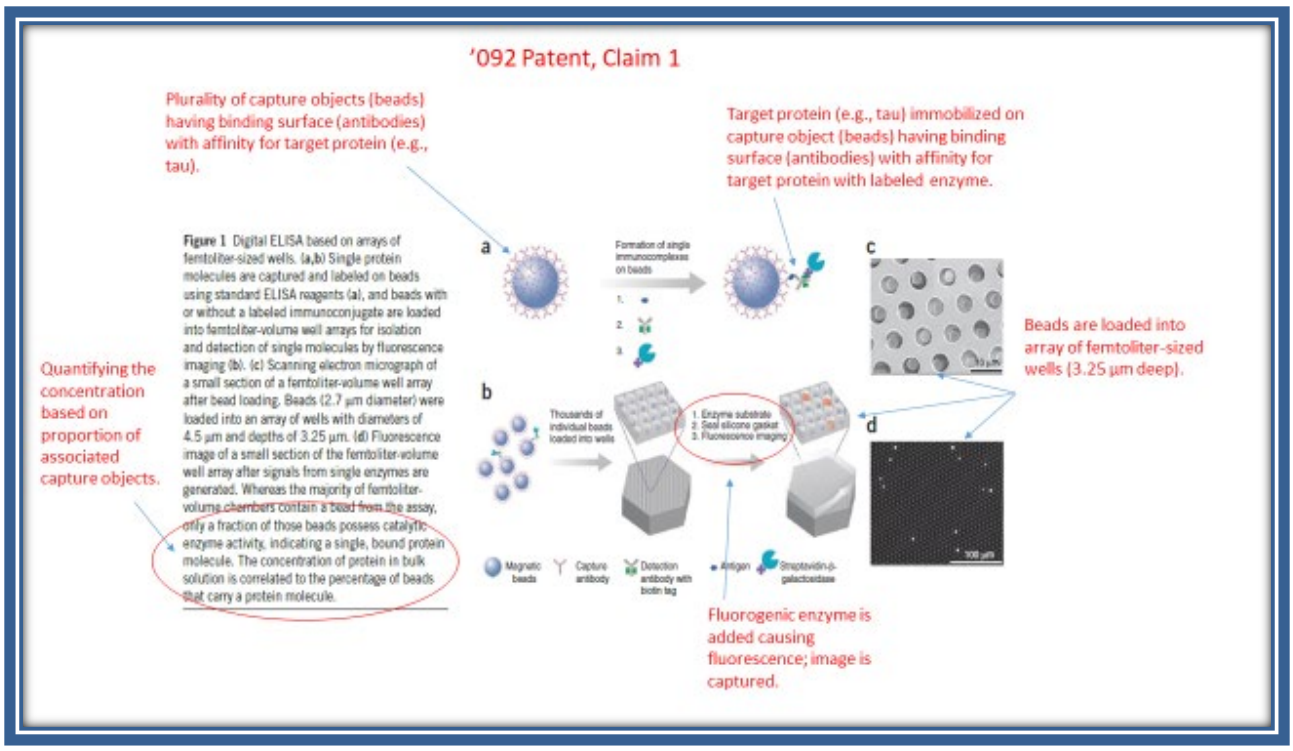


Figure 1 Digital ELISA based on arrays of femtoliter-sized wells. (**a**,**b**) Single protein molecules are captured and labeled on beads using standard ELISA reagents (**a**), and beads with or without a labeled immunoconjugate are loaded into femtoliter-volume well arrays for isolation and detection of single molecules by fluorescence imaging (**b**). (**c**) Scanning electron micrograph of a small section of a femtoliter-volume well array after bead loading. Beads (2.7 µm diameter) were loaded into an array of wells with diameters of 4.5 µm and depths of 3.25 µm. (**d**) Fluorescence image of a small section of the femtoliter-volume well array after signals from single enzymes are generated. Whereas the majority of femtoliter-volume chambers contain a bead from the assay, only a fraction of those beads possess catalytic enzyme activity, indicating a single, bound protein molecule. The concentration of protein in bulk solution is correlated to the percentage of beads that carry a protein molecule.

103. Rissin discloses that "[i]n the first step of this [SMA] (Fig. 1a), a sandwich antibody complex is formed on microscopic beads (2.7 μm diameter), and the bound complexes are labeled with an enzyme, as in a conventional bead-based ELISA." SMAs "permit the detection of very low concentrations of enzyme labels by confining the fluorophores generated by individual enzymes to extremely small volumes (~50 fl)…." To achieve this confinement, "beads are loaded into an array of femtoliter-sized wells." A "droplet of fluorogenic enzyme substrate", is added causing those beads "possessing a single enzyme–labeled immunocomplex" to fluoresce. "By acquiring time-lapsed fluorescence images of the array… it is possible to distinguish beads associated with a single enzyme molecule ("on" well) from those not associated with an enzyme ("off" well)." EX-1005, 596, Fig. 1.



EX-1005, 595; FIG. 1.

104. "Imaging the arrays allows simultaneous detection of tens to tens of thousands of single immunocomplexes. The protein concentration in the test sample is determined by counting the number of wells containing both a bead and fluorescent product relative to the total number of wells containing beads (Fig. 1d)" [*a concentration of protein in the volume of bodily fluid to produce the bodily fluid sample containing the analytically quantified amount of endogenous protein*]. EX-1005, 596. While Rissin does not expressly state that the protein is tau, Rissin cites to articles demonstrating that tau is known to be present in ultra-low concentrations in the blood of patients diagnosed with AD (e.g., citations 8

and 9) and, thus, directs the POSA to use Rissin's SMA to quantify blood tau levels as a biomarker for neurological disorders. EX-1005, 595. Moreover, Todd teaches performing SMA for the detection and diagnosis of AD and expressly identifies tau proteins as one of two "hopeful candidates" to serve as an FDA approved biomarker for clinical diagnosis of AD. EX-1006, 53. A POSA understood that Rissin's teaching equally applied to tau and would have been motivated to modify the teaching of Rissin with the teaching of Todd to quantify *a concentration of tau protein in the volume of bodily fluid* for the reasons discussed in the Motivation to Combine Section.

v. wherein the analytically quantified concentration of endogenous tau protein contained in the bodily fluid sample is less than about 5 pg/ml, and a limit of quantification of the analytical protein concentration measurement assay used in step (C) for quantifying the concentration of tau protein is less than about 0.2 pg/mL.

105. Rissin discloses this limitation. The "lowest concentration of enzyme conjugate detected" (LoQ) by Rissin was 350 zeptomolar (zM) and the calculated limit of detection (LoD) was 220 zM, corresponding to an increase in sensitivity over prior art measurements using a typical ELISA-plate reader "of a factor of about 68,000." EX-1005, 596; While Rissin does not expressly teach tau, as described in the

Motivation to Combine Section, Rissin does teach that an SMA provides sensitivities of 0.006 and 0.01 pg/mL using two of the same blood proteins studied in Todd, PSA and TNF-alpha, respectively, which are less than the claimed 0.2 pg/mL. EX-1005, 597-98. Moreover, Figure 2 of Rissin demonstrates a LoQ of the assay of less than 3.5 aM (with a coefficient of variance of 10%) when detecting the analyte streptavidin-β-galactosidase ("SBG"). A POSA understood that Rissin's teaching equally applied to tau and would have been motivated to modify the teaching of Rissin with the teaching of Todd to achieve analytically quantified concentration of endogenous tau less than about 5 pg/ml, and a limit of quantification of less than about 0.2 pg/mL for the reasons discussed in the Motivation to Combine Section

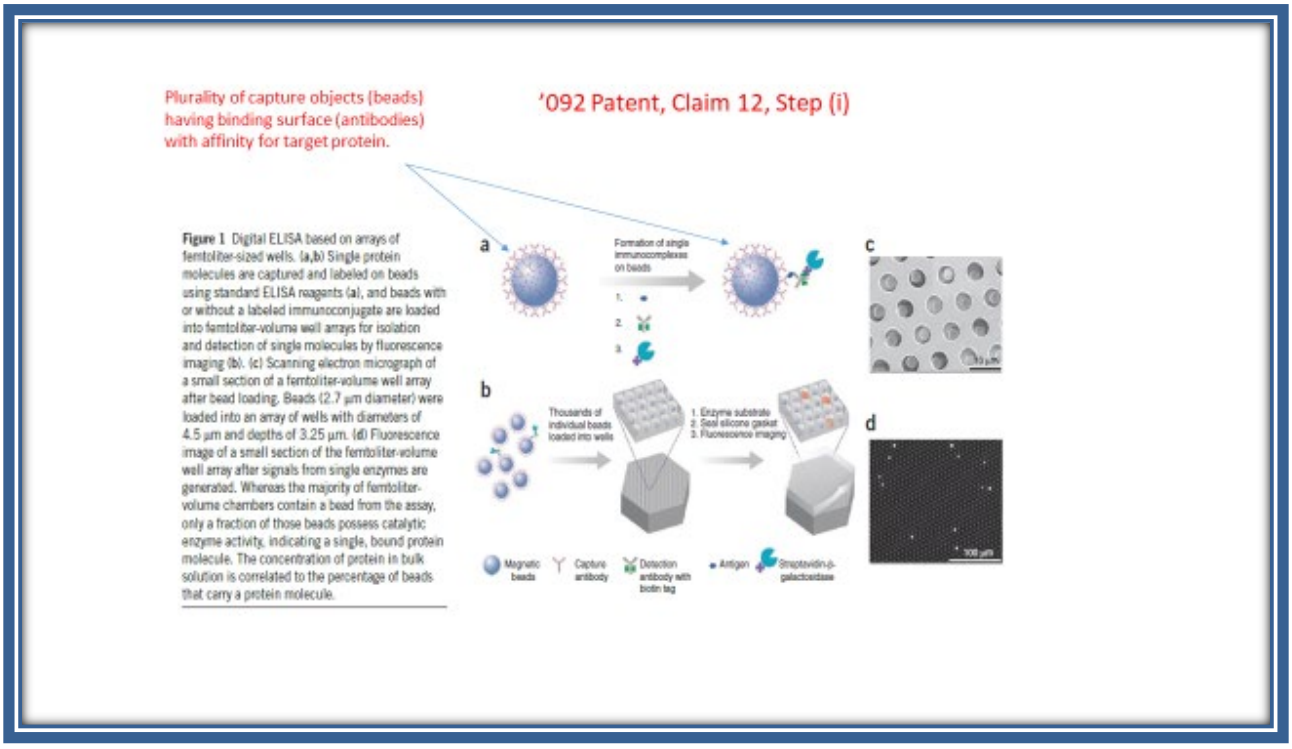
b. <u>Claim 12</u>

i. The method of claim 1, wherein the analytical protein concentration measurement assay used in step (C) comprises:

(a) i. suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of bodily fluid,

106. Rissin in view of Todd discloses this limitation. As shown in annotated Figure 1 below, Rissin discloses "[i]n the first step of this single-molecule immunoassay (Fig. 1a), a sandwich antibody complex

[*a binding surface having affinity for protein molecules*] is formed on microscopic beads (2.7 µm diameter) [*a plurality of capture objects*], and the bound complexes are labeled with an enzyme, as in a conventional bead-based ELISA." "Test solutions (100 µl) containing the protein of interest were incubated with suspensions of 200,000 magnetic beads… then separated and washed… re-suspended and incubated with solutions containing detection antibody… then separated and washed… [and] then re-suspended in 10 µl of PBS and loaded" [*suspending a plurality of capture objects*]. EX-1005, 596-97.



EX-1005, 595; FIG. 1.

107. Rissin discloses plurality of suspended beads [*capture objects*] having an affinity for the target protein (the illustrated antigen). While Rissin does not expressly disclose a capture antibody with an affinity for a tau, Rissin directs the POSA to use Rissin's SMA technology to quantify blood tau levels as a biomarker for neurological disorders.

108. Todd discloses a bead-based SMA in which a target protein (*e.g.*, tau) is immobilized on a plurality of "paramagnetic microparticles" (*i.e.*, a "capture objects") having affinity for target protein and suspended in a volume of a patient biological sample, such as blood serum or plasma. EX-1006, 53. Moreover, Todd teaches performing SMA for the detection and diagnosis of AD and expressly identifies tau proteins as one of two "hopeful candidates" to serve as an FDA approved biomarker for clinical diagnosis of AD. EX-1006, 53. As discussed in the State of the Art Section and in the specification of the 092 patent, capture antibodies with affinity for tau (anti-tau antibodies) were both well-known and commercially available in the art. EX-1001, 8:37-51; 33:34-40 (disclosing "commercial[ly] available antibody purchased from Covance").

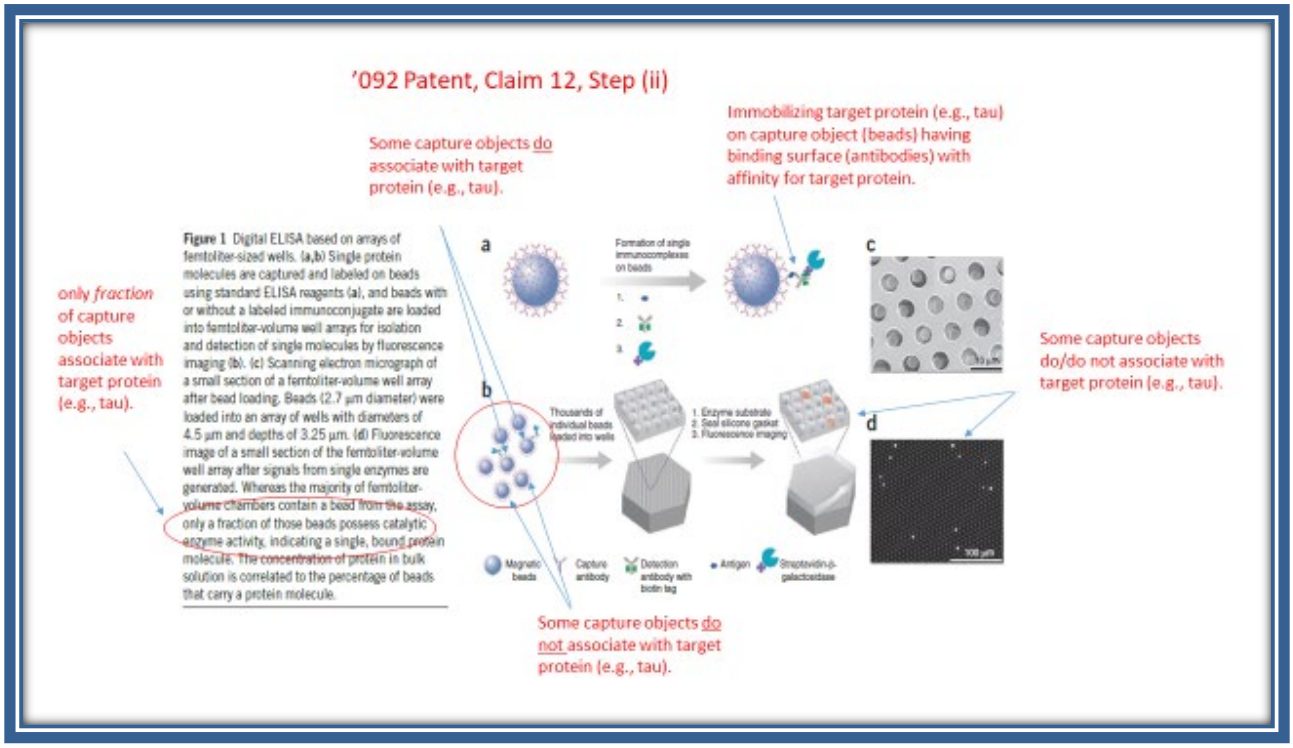
109. A POSA understood that Rissin teaches a SMA capable of detecting proteins at subfemtomolar concentrations, and would

have been motivated to use a capture antibody having an affinity for tau as disclosed in Todd for the reasons discussed in the Motivation to Combine Section.

(b) ii. immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of bodily fluid such that at least some of the capture objects associate with at least one tau protein molecule from the volume of bodily fluid,

110. Rissin as modified by Todd discloses this limitation. As demonstrated for limitation 12(i) above, in the first step of Rissin "(**Fig. 1a**), a sandwich antibody complex is formed on microscopic beads (2.7 μm diameter), and the bound complexes are labeled with an enzyme, as in a conventional bead-based ELISA." Those "beads are loaded into an array of femtoliter-sized wells for isolation and detection of single molecules by fluorescence imaging (b)." "Whereas the majority of femtoliter-volume chambers contain a bead from the assay, only a fraction of those beads possess catalytic enzyme activity, indicating a single, bound protein molecule" [*immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of bodily fluid such that at least some of*

the capture objects associate with at least one tau protein molecule from the volume of bodily fluid]. EX-1005, 596-97.



EX-1005, 595; FIG. 1.

111. For example, if 50 aM of protein in 0.1 ml (3,000 molecules) is captured and labeled on 200,000 beads, then 1.5% of the beads will carry one protein molecule and 98.5% will not carry any protein molecules (**Fig. 1b**). EX-1005, 595.

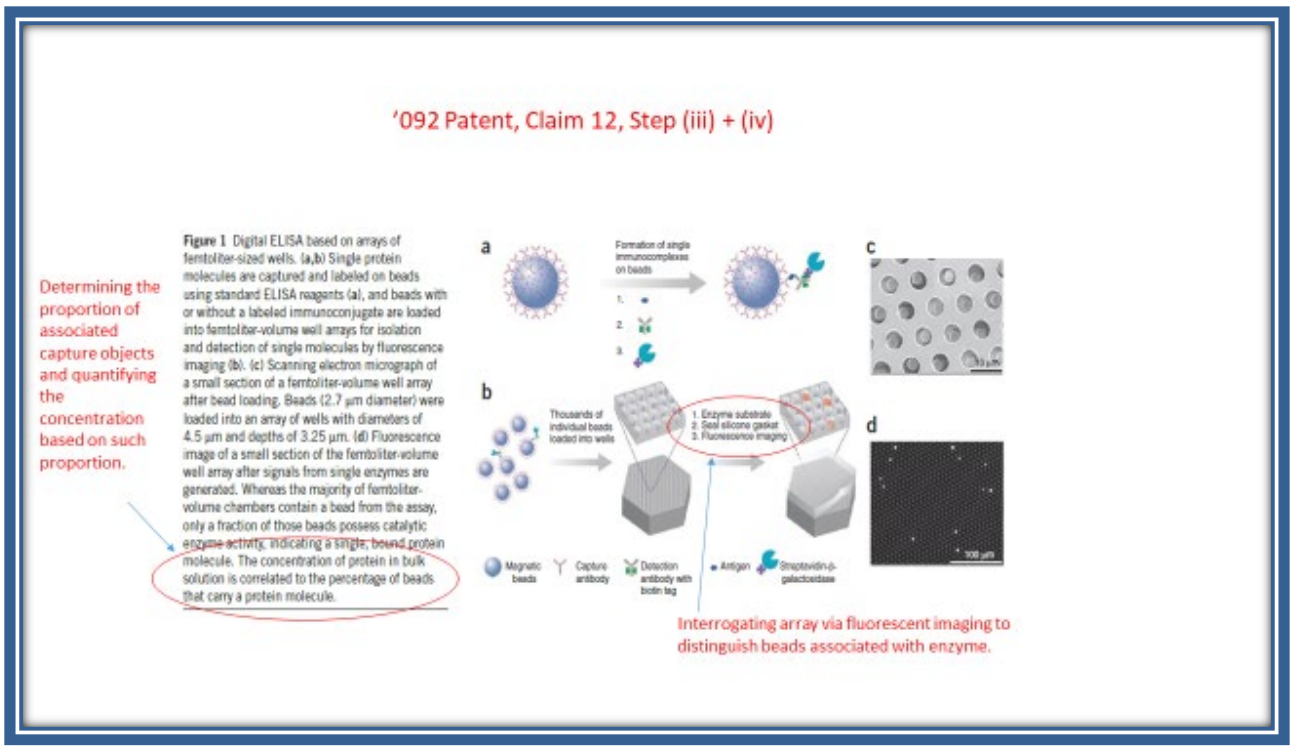
112. While Rissin does not expressly disclose tau, Rissin directs the POSA to use Rissin's SMA technology to quantify blood tau levels as a biomarker for neurological disorders. Moreover, Todd teaches performing a bead-based SMA for the detection and diagnosis of

AD and expressly identifies tau proteins as one of two "hopeful candidates" to serve as an FDA approved biomarker for clinical diagnosis of AD. EX-1006, 53. A POSA would understand that Rissin teaches SMA capable of detecting proteins at subfemtomolar concentrations, and would be motivated to use a capture antibody to immobilize at least a portion of tau as disclosed in Todd for the reasons discussed in the Motivation to Combine Section.

(c) iii. interrogating at least some of the capture objects associated with at least one tau molecule from the volume of bodily of fluid; and

iv. determining a measure of tau protein molecule concentration in the volume of bodily fluid based at least in part on the interrogating step performed in step iii.

113. Rissin, as modified by Todd to use a capture antibody having affinity for tau protein as discussed above, discloses this limitation. As illustrated in annotated FIG. 1 below, Rissin discloses a streamlined SMA in which the concentration of target analyte is determined directly by interrogating the relative proportion of capture complexes in the array.



EX-1005, 595; FIG. 1.

114. Using time-lapsed fluorescence images, Rissin "detects single protein molecules" on the captured complex itself (the same objects exposed to an analyte-containing solution and immobilized with analyte molecules or particles) in order to distinguish "beads associated with a single enzyme molecule ("on" well) from those not associated with an enzyme ("off" well)" [*interrogating at least some of the capture objects associated with at least one tau molecule from the volume of bodily of fluid*]. Finally, "[t]he protein concentration in the test sample is determined by counting the number of wells containing both a bead and fluorescent product relative to the total number of wells containing beads

(Fig. 1d)" [*determining a measure of tau protein molecule concentration in the volume of bodily fluid based at least in part on the interrogating step performed in step iii*]. EX-1005, 595-599 and FIGs. 1, 2; Supp. FIGs 1, 2.

ii. **<u>Claim 13</u> - *The method of claim 12, wherein the plurality of capture objects comprises a plurality of beads.***

115. Both Rissin in view of Todd teach that the capture objects comprise a plurality of beads and, thus, disclose this limitation for the same reasons discussed for Claim 12.

c. <u>Claim 6</u> – *The method of claim 1, wherein each of the tau protein molecules quantified in step (C) is selected from the group consisting of tau 23 (352, 0N3R), tau 2.4 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R).*

116. Rissin as modified by Todd discloses this limitation. Claim 6 recites a specific subset of isoforms that make up total tau, including tau 23 (352, 0N3R), tau 2.4 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R). As shown above, Todd expressly teaches the detection of "tau proteins" in a human blood samples, which a POSA understood to include the specific subset of isoforms that make up total tau recited in claim 6, including phosphorylated tau (claim 7). EX-1006, 53. Moreover, as discussed in the State of the Art Section and in the specification of the '092 Patent, the

"six known natural-occurring tau proteins" are "well-known in the art", including "phosphorylated" tau. EX-1001, 8:37-51.

117. As such, a POSA understood that Rissin as modified by Todd would include quantification of each of the identified the isoforms of tau recited in claim 6.

d. <u>Claim 7</u> – *The method of claim 6, wherein at least a portion of the tau protein molecules are phosphorylated.*

118. Rissin as modified by Todd discloses this limitation for the same reasons discussed for claim 6. EX-1006, 53; EX-1001, 8:37-51. Moreover, as set forth in the State of the Art, a POSA understood that under normal physiological conditions, tau functions to promote the assembly and stabilization of neuron structure. However, in AD and other neurodegenerative diseases, tau becomes abnormally phosphorylated, causing it to detach and aggregate into tangled fibers called neurofibrillary tangles. A POSA understood that phosphorylated tau was a blood biomarker for neurodegenerative diseases, including TBI. EX-1022, 9916.

119. As such, a POSA understood that tau serum marker discussed in Todd would include phosphorylated tau.

e. <u>Claims 8-11</u> – *The method of claim 1, wherein the volume of bodily fluid comprises blood (claim 8), plasma (claim 9), serum (claim 10) or not cerebrospinal fluid (claim 11).*

120. Rissin teaches a SMA that "detects low-abundance proteins in blood" [claim 8] and "serum proteins at subfemtomolar concentrations" [claim 10]. EX-1005, 595-599. While Rissin does not expressly disclose the detection of such proteins in blood "plasma", a POSA understood that Rissin's disclosure of "blood" would include all of its constituent components, including both blood plasma [claim 9], and would not include cerebrospinal fluid [claim 11]. Moreover, Rissin cites to articles concerning the "plasma proteome" for support (citation 19), showing that Rissin's disclosure of "blood" clearly directed the POSA to all constituent components of blood. A POSA was have understood Rissin to teach a method of producing a bodily fluid comprising blood [claim 8], plasma [claim 9], serum [claim 10] or not cerebrospinal fluid [claim 11]. EX-1005, 595-599.

f. <u>Claim 14</u> – *The method of claim 1, wherein the limit of detection for the analytical protein concentration measurement assay is less than about 0.02 pg/mL.*

121. Rissin teaches SMA having a LoD of less than about 0.02 pg/ml. In particular, Rissin teaches a "LoD of ~50 aM (1.5 fg/ml)" when detecting PSA (*e.g.*, Prostate-Specific Antigen) in 25% serum, "which equates to an LoD in whole serum of ~200 aM (6 fg/ml).

The lowest concentration tested and detected was 250 aM in 25% serum, corresponding to 1 fM in whole serum." EX-1005, 597.

g. <u>Claim 15</u> – *The method of claim 1, wherein the patient suspected of having a neurological condition is recovering from a brain injury.*

122. Claim 15 purports to limit the "neurological condition" recited in the preamble of claim 1 to that of a "brain injury". As Claim 15 does not alter the steps to be performed in the claimed method, to the extent it is considered a limitation, it is nonetheless disclosed for the same reasons discussed for the preamble of claim 1 and as further understood from the State of the Art. EX-1027, cls 1, 2, 4; EX-1018, 19-20.

123. Consistent with the discussion in the State of the Art Section (including the Quanterix Presentation presented by PO), a POSA understood that brain injuries are linked to causing neurological disorders, such as AD, and that tau was a known indicator of TBI from a hypoxic or ischemic event, including cardiac arrest, stroke or thrombosis. EX-1027, cls 1, 2, 4; EX-1018 at 19-20; EX-1033, slides 28-29 (tau is "a nice serum marker for TBI"). Thus, for the reasons set forth for claim 1, claim 15 would have been obvious over Rissin in view of the State of the Art.

h. Claims 17-21 – *The method of claim 15, wherein the brain injury results from a hypoxic event (claim 17), caused by cardiac arrest (claim 18), stroke (claim 19), an ischemic event (claim 20), or thrombosis (claim 21)*

124. Claims 17-21 purport to limit the "neurological condition" recited in the preamble of claims 1 and 15 to that of a "brain injury" resulting from a "hypoxic event" [claim 17] or ischemic event [claim 20], such as cardiac arrest [claim 18], stroke [claim 19], or thrombosis [claim 21]. Like Claim 15, the recited limitations of claims 17-21 do not alter the steps to be performed in the claimed method. Moreover, a POSA understood that brain injury can result from any type of hypoxic or ischemic event, including the specific events of cardiac arrest, stroke or thrombosis, and that tau is an effective biomarker for brain injury in the blood. To the extent these specific indications are considered limitations, however, they are disclosed for the same reasons discussed as to the preamble of claim 1 as understood from the State of the Art. EX-1027, cls 1, 2, 4; EX-1017, 559, 563, and Fig. 1b; EX-1018, 19-20.

i. Claim 16 - *The method of claim 15, wherein the volume of bodily fluid has been collected from the patient within 12 hours of the brain injury.*

125. Rissin as modified by Todd discloses this limitation. As set forth in the State of the Art, a POSA understood the prior art taught that elevated tau could be detected in blood of acute

ischemic stroke patients at levels of 30 pg/ml at 3, 6 and 12 hours after stroke, and that tau was a predictor of outcome in stroke patients. EX-1017, 559, 563, and Fig. 1b. Given the increase sensitivity of SMAs, it would have been obvious to a POSA at the time of the invention to test tau blood levels at intervals immediately following a brain injury, including within 12 hours of such an event, and a POSA would have had a reasonable expectation of success in doing so. Moreover, given the known prognostic benefits of early disease detection, a POSA would have been motivated to begin testing tau levels at intervals immediately following a brain injury, including within 12 hours of such an event.

B. Challenge 2: Claims 1-21 of the '092 Patent are invalid as obvious over Rissin in view of Voorheis

126. Voorheis is directed to methods and kits for diagnosing AD and other neurological disorders by screening for tau in the blood of a patient. The invention is based, in part, on the discovery that "proteolytic fragments of… tau-proteins are released from the neurofibrillary tangles associated with the disease and can be detected in body fluids outside the brain." Voorheis notes that it was "well documented" that "the number and size of these tangles within an affected neuron as well as the total number of tangles and the total amount of

constituent tau-protein in an affected brain correlates with the progression and severity of the disease." EX-1007, 4:10-15, 5:19-23; 6:57-67.

127. Voorheis discussed the inability of prior art methods to readily and reliably diagnose AD, and the need for sensitive diagnostic tests to screen body fluids of individuals for the presence of tau biomarkers. Prior art methods focused on evaluating the patient's intellectual ability: "[u]sually a patient must be evaluated on a number of occasions over some period of time in order to document the deterioration in intellectual ability and other signs and symptoms before a diagnosis can be attempted with reasonable confidence." "The necessity of repeated evaluation… before a firm diagnosis [could] be made was costly, generates anxiety and… frustrating to patients and their families." Furthermore, the inability to rapidly diagnose the disease "greatly hampered" the development of a therapeutic strategy, "particularly in the early stages where even a simple arrest of the progress of the disease would leave the patient with significant intellectual capacity and a reasonable quality of life." Thus, Voorheis recognized the need for early detection and diagnosis of AD, and developed a blood-based diagnostic assay using tau as a method for doing so. EX-1007, 3:4-28.

128. "In practicing the invention, a sample of blood is removed from the patient by venesection…." The sample is then "diluted in an appropriately buffered medium". In one mode of the invention, tau is "used as antigens in immunoassays for the detection of those individuals suffering from Alzheimer's." These immunoassay systems include "any known in the art, including but not limited to: radioimmunoassays, [ELISA], 'sandwich' assays, precipitin reactions, gel diffusion immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays, to name but a few." Voorheis notes that, "[p]articularly preferred, for ease of detection, and its quantitative nature, is the sandwich or double antibody assay, of which a number of variations exist." EX-1007, 13:35-60, 14:5-31, 16:1-6.

129. Voorheis recognized that tau could broadly include "any one of the proteins that comprise a single constituent part of the tau-complex of proteins." Because "concentrations [of tau] greatly exceed[] that found in normal humans that are not victims of Alzheimer's", the "detection and quantitation" of tau "is useful in confirming a clinical diagnosis of Alzheimer's… and in following the course of the disease." Tau concentration "may be calculated in absolute terms, or may be done in comparison with a standard (or series of standards) containing a known

normal level of antigen." Moreover, Voorheis recognized that "other brain-specific, soluble and protease-sensitive proteins find their way into blood and spinal fluid following head trauma… and various other types of neurological disorders." EX-1007, 5:19-56, 7:1-18, 14:60-65.

1. The Motivation to Combine Rissin in view of Voorheis

130. A POSA would have been motivated to combine the teachings of Rissin with the teachings of Voorheis in order to quantify endogenous tau in the blood of patients suspected of having a neurological disorder as claimed by the '092 Patent for several reasons.

131. Rissin and Voorheis are analogous art as they are in the same field of endeavor as the '092 Patent. Both references are directed at detecting and quantifying low concentrations of analytes in the blood of patients for purposes of detection and diagnosis of neurological diseases.

132. A POSA understood that a simple, easy to use and ultra-sensitive technique to detect trace amounts of analyte in blood would have been beneficial for early detection and diagnosis of neurological disorders. Rissin expressly states that the "isolation and detection of single protein molecules [using SMA] provides a promising approach for measuring extremely low concentrations of proteins… using the same reagents as the gold standard for detecting proteins, namely, the ELISA"

but now "at subfemtomolar concentrations." Rissin notes that "serum concentrations of the majority of proteins important in… neurological disorders… are thought to range from 10^{-16} to 10^{-12} M" and, in support, cites to two articles, EX-1025, EX-1029, discussing tau protein as a blood biomarker for AD. Thus, a POSA reading Rissin understood that tau was both present at low concentrations in blood, and was a viable candidate for quantification using SMA. EX-1005, 595.

133. A POSA also understood that tau was recognized as a biomarker in human CSF, blood and blood components for early diagnosis and prognostic prediction of the changes in brain function and other cognitive disorders. Voorheis developed methods and kits for diagnosing neurological disorders, including AD, by screening for tau in human blood. A POSA understood that the earlier tau can be detected, the sooner a clinician can diagnosis, and begin treatment of, a neurological condition. Voorheis emphasized the need for sensitive diagnostic tests to screen body fluids for the presence of tau biomarkers, "particularly in the early stages where even a simple arrest of the progress of the disease would leave the patient with significant intellectual capacity and a reasonable quality of life." EX-1007, 3:23-28.

134. Armed with this extensive knowledge of tau and SMA, a POSA as of the priority date would have been motivated to test the blood of a patient suspected of having a neurological disorder to detect and quantify the presence of endogenous tau using ultra-sensitive SMA, and would have a reasonable expectation of success in doing so.

135. ***First***, a POSA understood AD, a progressive neurodegenerative disorder that effects more than 6 million Americans, costs hundreds of billions of dollars annually in long-term care, is the most common cause of dementia in older adults, and is the seventh leading cause of death in the US. Voorheis recognized market pressure to solve the problem of early AD diagnosis, and taught assaying blood for tau to address the problem. Tau in blood as taught to be detected by Voorheis to diagnose AD could be readily and reliably quantified using Rissin's SMA assay.

136. ***Second***, given that SMA assays were simple, convenient, and inexpensive to conduct and provided ultra-high sub-femtomolar analyte sensitivity, selecting Rissin's SMA assay that was both more sensitive than conventional ELISA and easy to use was a matter of common sense, especially when seeking to detect analytes known to be present in very low concentrations in blood such as tau. This is

particularly true when combined with the fact that a POSA recognized that tau concentration correlates with disease progression and, therefore, the sooner tau could be detected, the sooner the condition could be clinically treated.

137. As such, the published prior art provided all the data a POSA would need to try – with a reasonable expectation of success – a method of quantifying endogenous tau in blood of patients suspected of having a neurological disorder as taught by Voorheis, using the SMA taught by Rissin, within the scope of the '092 patent – using the "digital ELISA" (SMA) of Rissin in place of ELISA of Voorheis.

2. Detailed Application of Rissin in view of Voorheis

a. <u>Claim 1</u>

i. A method of producing a bodily fluid sample of a patient suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein, said method comprising:

138. To the extent the preamble is a limitation, Rissin discloses a method for performing simplified SMA to quantify concentrations of protein in blood for clinical diagnosis of neurological disease. EX-1005, 596. Rissin does not expressly state that the protein could be tau, Rissin's citation to articles demonstrating that tau is known to

be present in ultra-low concentrations in the blood of patients diagnosed with AD (e.g., citations 8 and 9) directs the POSA to use Rissin's SMA technology to quantify blood tau levels as a biomarker for neurological disorders. EX-1005, 595. Moreover, Voorheis teaches that tau could be measured in blood and blood plasma and used as a diagnostic marker for neurological disorders, including AD. EX-1007, Abstract. A POSA would have been motivated to modify the teachings Rissin with the teachings of Voorheis in order to quantify protein concentrations in blood for clinical diagnosis of neurological disease for the reasons discussed in the Motivation to Combine Section. This modification results in a *method of producing a bodily fluid sample of a patient suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein*, consistent with the State of the Art that a POSA understood tau was a known indicator of TBI resulting from a hypoxic or ischemic event, including cardiac arrest, stroke or thrombosis. EX-1027, cls 1, 2, 4; EX-1018 at 19-20.

ii. (A) obtaining a volume of bodily fluid comprising blood, or a blood component selected from plasma and serum,

139. With references to the discussion of Rissin in Ground 1 above, a POSA reading Rissin understood that it includes the

step of obtaining a volume of body fluid comprising blood, or a blood component selected from plasma and serum. EX-1005, 1-2.

140. Voorheis expressly discloses that "[i]n practicing the invention, a sample of blood is removed from the patient by venesection", which a POSA understood included blood components including plasma and serum [*obtaining a volume of bodily fluid comprising blood*]. EX-1007, 13:55-60.

iii. (B) diluting the volume of bodily fluid, and

141. With references to the discussion of Rissin in Ground 1 above, Rissin discloses this limitation. Rissin discloses that whole-serum samples collected for use in SMA arrays "were diluted 1:4 in buffer and measured using the digital ELISA specific for PSA (Fig. 3a)" [*diluting the volume of bodily fluid*]**.** EX-1005, 596-598; Fig. 3a (teaching "25% serum").

iv. (C) quantifying through the use of an analytical protein concentration measurement assay a concentration of tau protein in the volume of bodily fluid to produce the bodily fluid sample containing the analytically quantified amount of endogenous tau protein;

142. Rissin in view of Voorheis discloses this limitation. As discussed in Ground 1 above, Rissin makes use of SMAs that "can

isolate and detect single enzyme molecules" [*quantifying through the use of an analytical protein concentration measurement assay*] and teaches that the "protein concentration in the test sample is determined by counting the number of wells containing both a bead and fluorescent product relative to the total number of wells containing beads (Fig. 1d)" [*a concentration of protein in the volume of bodily fluid to produce the bodily fluid sample containing the analytically quantified amount of endogenous protein*]. EX-1005, 596, Fig. 1.

143. While Rissin does not expressly disclose tau, Rissin directs the POSA to use Rissin's SMA technology to quantify blood tau levels as a biomarker for neurological disorders. A POSA understood that Rissin's teaching equally applied to tau and would have been motivated to modify the teaching of Voorheis to quantify *a concentration of tau protein in the volume of bodily fluid* for the reasons discussed in the Motivation to Combine Section.

v. wherein the analytically quantified concentration of endogenous tau protein contained in the bodily fluid sample is less than about 5 pg/ml, and a limit of quantification of the analytical protein concentration measurement assay used in step (C) for quantifying the concentration of tau protein is less than about 0.2 pg/mL.

144. As discussed in Ground 1 above, Rissin discloses this limitation. EX-1005, 596 (disclosing a calculated LOD of 220 zM). While Rissin does not expressly teach tau, as described in the Motivation to Combine Section, Rissin does teach that an SMA provides sensitivities of 0.006 and 0.01 pg/mL for two proteins PSA and TNF-alpha, in serum, respectively, which are less than the claimed 0.2 pg/mL. Moreover, Figure 2 of Rissin demonstrates a LoQ of the assay of less than 3.5 aM (with a coefficient of variance of 10%) when detecting the analyte streptavidin-β-galactosidase ("SBG"). A POSA understood that Rissin's teaching equally applied to tau and would have been motivated to modify the teaching of Rissin with the teaching of Voorheis to achieve analytically quantified concentration of endogenous tau less than about 5 pg/ml, and a limit of quantification of less than about 0.2 pg/mL for the reasons discussed in the Motivation to Combine Section.

b. <u>Claim 12</u>

i. The method of claim 1, wherein the analytical protein concentration measurement assay used in step (C) comprises:

145. Claim 12 is disclosed by Rissin as modified by Voorheis and a POSA would be motivated to use the simplified SMA

assay of Rissin to quantity the concentration of tau disclosed in Voorheis for the reasons discussed in Motivation to Combine Section.

(a) i. suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of bodily fluid,

146. As discussed in Ground 1 above, Rissin discloses this limitation. Rissin discloses a sandwich antibody complex [*a binding surface having affinity for protein molecules*] formed on microscopic beads [*a plurality of capture objects*] "…and incubated with solutions containing detection antibody… then separated and washed… [and] then re-suspended in 10 µl of PBS and loaded" [*suspending a plurality of capture objects*]. Thus, Rissin discloses plurality of suspended beads [*capture objects*] having an affinity for the target protein (the illustrated antigen), but does not expressly disclose a capture antibody with an affinity for tau.

147. However, Voorheis teaches the use of "a number of antibodies [that are] described in the literature as 'anti-tau'" including "commercial antibody described as 'anti-tau'", as well as numerous "procedures known in the art" that can be "used for the production of antibodies to epitopes of the tau-protein." EX-1007, 6:10-35; 12:35-13:31. A POSA understood that Rissin teaches a SMA capable of detecting proteins at subfemtomolar concentrations, and would have been motivated

to use a capture antibody having an affinity for tau as disclosed in Voorheis for the reasons discussed in the Motivation to Combine Section.

(b) ii. immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of bodily fluid such that at least some of the capture objects associate with at least one tau protein molecule from the volume of bodily fluid,

148. Rissin as modified by Voorheis discloses this limitation for the same reasons discussed in connection with Claim 12(b) in Ground 1.

(c) iii. interrogating at least some of the capture objects associated with at least one tau molecule from the volume of bodily of fluid; and

149. Rissin as modified by Voorheis discloses this limitation for the same reasons discussed in connection with Claim 12(c) in Ground 1.

(d) iv. determining a measure of tau protein molecule concentration in the volume of bodily fluid based at least in part on the interrogating step performed in step iii.

150. Rissin as modified by Voorheis discloses this limitation for the same reasons discussed in connection with Claim 12(d) in Ground 1.

ii. **Claim 13** - ***The method of claim 12, wherein the plurality of capture objects comprises a plurality of beads.***

151. Rissin discloses this limitation for the same reasons discussed for Claim 12.

c. **Claim 6** – ***The method of claim 1, wherein each of the tau protein molecules quantified in step (C) is selected from the group consisting of tau 23 (352, 0N3R), tau 2.4 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R).***

152. Rissin as modified by Voorheis discloses this limitation. Claim 6 recites a specific subset of isoforms that make up total tau, including tau 23 (352, 0N3R), tau 2.4 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R). Voorheis teaches that tau proteins broadly include "any one of the proteins that comprise a single constituent part of the tau-complex of proteins" which a POSA understood includes measurement of the isoforms of tau recited in claim 6, including phosphorylated tau (claim 7). EX-1007, 5:54-56.

d. **Claim 7** – ***The method of claim 6, wherein at least a portion of the tau protein molecules are phosphorylated.***

153. Rissin as modified by Voorheis disclose this limitation for the same reasons discussed for claim 6. Voorheis teaches that tau proteins broadly include "any one of the proteins that comprise a

single constituent part of the tau-complex of proteins" and describes that "inappropriate phosphorylation" of tau is associated with neurological diseases, including AD. EX-1007, 2:1-25.

154. Moreover, as set forth in the State of the Art, a POSA understood that under normal physiological conditions, tau functions to promote the assembly and stabilization of neuron structure. However, in AD and other neurodegenerative diseases, tau becomes abnormally phosphorylated, causing it to detach and aggregate into tangled fibers called neurofibrillary tangles. A POSA understood that phosphorylated tau was a blood biomarker for neurodegenerative diseases. EX-1022, 9916;

155. A POSA understood that Rissin teaches a SMA capable of detecting proteins at subfemtomolar concentrations, and would have been motivated to use Rissin's SMA to detect phosphorylated tau as taught by Voorheis for the reasons discussed in the Motivation to Combine Section.

e. <u>Claims 8-11</u> – *The method of claim 1, wherein the volume of bodily fluid comprises blood (claim 8), plasma (claim 9), serum (claim 10) or not cerebrospinal fluid (claim 11).*

156. With reference to Claims 8-11 in Ground 1 above, Rissin teaches a SMA that "detects low-abundance proteins in blood

[claim 8], plasma [claim 9], serum [claim 10] or not cerebrospinal fluid [claim 11]. EX-1005, 595-599.

157. Voorheis' diagnostic assays are based on the principle that cleaved "segments of tau-proteins that can find their way into body fluids outside the brain and whose detection and quantitation are important for diagnostic purposes." Voorheis discloses the detection of tau in "on samples of blood, spinal fluid or other bodily fluid". EX-1007, 13:50-55. More specifically, Voorheis teaches evaluating the amount of antigen present in "blood" (EX-1007, 13:50-55), "plasma" (EX-1007, 13:56-62) and "serum" (EX-1007, 14:66-15:25).

f. <u>Claim 14</u> – *The method of claim 1, wherein the limit of detection for the analytical protein concentration measurement assay is less than about 0.02 pg/mL.*

158. With reference to claim 14 in Ground 1 above, Rissin teaches a SMA wherein the LOD for the analytical protein concentration measurement assay is "~50 aM (1.5 fg/ml)", which is less than about 0.02 pg/mL. EX-1005, 597.

g. <u>Claim 15</u> – *The method of claim 1, wherein the patient suspected of having a neurological condition is recovering from a brain injury.*

159. With reference to claim 15 in Ground 1 above, limiting the "neurological condition" recited in the preamble of claim 1 to

that of a "brain injury" does not alter the steps to be performed in the claimed method. To the extent it is considered a limitation, however, it is nonetheless disclosed for the same reasons discussed in the preamble of claim 1 and as further described in the State of the Art. EX-1027, cls 1, 2, 4; EX-1018, 19-20.

160. Consistent with the discussion in the State of the Art Section (including the Quanterix Presentation presented by Patent Owner), a POSA understood that brain injuries are linked to causing neurological disorders, such as AD, and that tau was a known indicator of TBI from a hypoxic or ischemic event, including cardiac arrest, stroke or thrombosis. EX-1027, cls 1, 2, 4; EX-1018 at 19-20; EX-1033, slides 28-29 (tau is "a nice serum marker for TBI"). Thus, for the reasons set forth for claim 1, claim 15 would have been obvious over Rissin in view of the State of the Art.

161. In addition, Voorheis is directed to the diagnosis, subtyping and monitoring of AD, which a POSA understood is a type of brain injury. EX-1007 1:10-25.

h. <u>Claims 17-21</u> – *The method of claim 15, wherein the brain injury results from a hypoxic event (claim 17), caused by cardiac arrest (claim 18), stroke (claim 19), an ischemic event (claim 20), or thrombosis (claim 21).*

162. With reference to claims 17-21 in Ground 1 above, limiting the "neurological condition" recited in the preamble of claims 1 and 15 to that of a "brain injury" resulting from a "hypoxic event" [claim 17] or ischemic event [claim 20], such as cardiac arrest [claim 18], stroke [claim 19], or thrombosis [claim 21] do not alter the steps to be performed in the claimed method. Moreover, a POSA understood that brain injury can result from any type of hypoxic or ischemic event, including the specific events recited in claims 17-21, and that tau is an effective biomarker for brain injury in the blood. To the extent these specific indications are considered limitations, however, they are disclosed for the same reasons discussed in the preamble of claim 1 as further described in the State of the Art. EX-1027, cls 1, 2, 4; EX-1017, 559, 563, and Fig. 1b; EX-1018, 19-20.

i. <u>Claim 16</u> - *The method of claim 15, wherein the volume of bodily fluid has been collected from the patient within 12 hours of the brain injury.*

163. Rissin in view of Voorheis renders this limitation obvious. Rissin teaches a diagnostic SMA with increased sensitivity and describes the known prognostic benefits of "earlier diagnosis and treatment of disease." Similarly, Voorheis emphasized the need for sensitive diagnostic tests to screen body fluids for the presence of tau, "particularly

in the early stages where even a simple arrest of the progress of the disease would leave the patient with significant intellectual capacity and a reasonable quality of life." EX-1007, 3:23-28.

164. As set forth in the State of the Art, a POSA understood from the prior art that elevated tau could be detected in blood of acute ischemic stroke patients at levels of 30 pg/ml at 3, 6 and 12 hours after stroke, and that tau was a predictor of outcome in stroke patients. EX-1017, 559, 563, and Fig. 1b. Thus, in view of the increase sensitivity of SMAs and the known prognostic benefits of early disease detection, it would have been obvious to a POSA at the time of the invention to quantify tau blood concentration levels at intervals immediately following a brain injury, including within 12 hours of such an event, and a POSA would have been motivated to do so.

CONCLUSION AND DECLARATION

My findings and opinions set forth in this Declaration are based on my work and examination to date. I may continue my examinations in view of additional documents or other factual evidence over the course of this proceeding that may necessitate supplementing and/or refining my opinions. I reserve the right to add to, alter, or delete my opinions and my Declaration upon discovery of additional information. I reserve the right to make such changes as necessary.

In signing this Declaration, I understand that the Declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code.

[SIGNATURE ON FOLLOWING PAGE]

Date: 5/28/25 By: */s/John Todd, Ph.D*
John Todd, Ph.D

APPENDIX A

Exhibit #	***Description***
1001	U.S. Patent No. 11,275,092 ("the '092 Patent")
1002	Select portions of prosecution history of the '092 Patent, i.e., select portions of the file history of US application No. 16/522,237 ("File History")
1003	Intentionally Left Blank
1004	Intentionally Left Blank
1005	Rissin et al., Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 28(6):595-9 and Supplementary information (4 pages) available at https://static-content.springer.com/esm/art%3A10.1038%2Fnbt.1641/MediaObjects/41587_2010_BFnbt1641_MOESM21_ESM.pdf (Epub May 23, 2010) ("Rissin")
1006	John Todd, et al., How Low Can You Go? Next generation immunoassay systems and the revival of protein biomarkers. Drug Discovery World, Fall 2008 ("Todd") available at https://www.ddw-online.com/media/32/fall-08-immunoassay-systems.pdf. ("Todd")
1007	Voorheis, "Diagnostic Method For Alzheimer's Disease By Screening For Tau-Peptides in the Blood of a Patient", US Patent No. 5,492,812 ("Voorheis")
1008	File History of US application No. 08/159,969, filed November 30, 1993, which matured into US Patent No. 5,492,812 (Voorheis, EX-1011), and published February 20, 1996 with the issuance of Voorheis, EX-1007 ("Voorheis FH") (An electronic copy (pdf) is supplied with the filing of this Petition, and Petitioner also has a Certified Copy of Voorheis FH from the National Archives, available for filing by hand with the Board)
1009	Rissin & Walt, "Digital Concentration Readout of Single Enzyme Molecules Using Femtoliter Arrays and Poisson Statistics", Nano Lett. 6(3): 520-523 (2006) ("Rissin 2006")

Exhibit #	***Description***
1010	Rissin et al., "Simultaneous Detection of Single Molecules and Singulated Ensembles of Molecules Enable Immunoassays with Broad Dynamic Range", Anal. Chem. 83:2279-2285 (2011) ("Rissin 2011")
1011	Bourzac, "Next-Generation Diagnostics A startup can detect tiny traces of cancer markers in blood samples", MIT Technology Review, May 13, 2008, available at: https://www.technologyreview.com/2008/05/13/220548/next-generation-diagnostics/
1012	Todd et al., "Ultrasensitive Flow-based Immunoassays Using Single-Molecule Counting", Clinical Chemistry 53(11):1990-1995 (2007) ("Todd 2007")
1013	Nam et al., "Nanoparticle-Based Bio-Bar Codes for the Ultrasensitive Detection of Proteins, Science 301: 1884-1886 (2003) ("Nam")
1014	Thaxton et al., "Nanoparticle-based bio-barcode assay redefines 'undetectable' PSA and biochemical recurrence after radial prostatectomy", PNAS 44:18437-18442 (2009)
1015	Duffy et al., US2010/0075407 ("the '407 publication" or "Duffy"), published March 25, 2010
1016	Rissin et al., "Digital Readout of Target Binding with Attomole Detection Limits via Enzyme Amplification in Femtoliter Arrays", JACS Communications 19(128) 6286-6287 (2006) ("Rissin 2006a")
1017	Wunderlich et al., "Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke", Clinical Neurology and Neurosurgery 108:558-63 (Sep. 2006) ("Wunderlich")
1018	Mortberg, "Assessment of the Cerebral Ischemic/Reperfusion Injury after Cardiac Arrest", Acta Universitatis Upsaliensis Uppsala. Dissertation, 71 pages (2010) ("Mortberg")
1019	Ding et al., Transl Neurodegener 10:10 (2021). https://doi.org/10.1186/s40035-021-00234-5 ("Ding")
1020	Medeiros, et al., "The Role of Tau in Alzheimer's Disease and Related Disorders", CNS Neuroscience & Therapeutics 17: 514–524 (2011) ("Medeiros")
1021	Weingarten et al., "A protein factor essential for microtubule assembly", PNAS 72:1858-1862 (1975) ("Weingarten")

Exhibit #	***Description***
1022	Konzack et a., "Swimming against the Tide: Mobility of the Microtubule Associated Protein Tau in Neurons", J Neuroscience 27(37):9916 –9927l (2007)
1023	Vanmechelen et al., "Differential Diagnosis of Neurological Diseases", US Patent No. 6,670,137 ("Vanmechelen")
1024	April 9, 2004 "INNOTEST hTAU Ag" webpage of Innogenetics downloaded from "Wayback Machine", https://web.archive.org/web/20040914040003fw_/http://www.innogenetics.be/site/prodview.asp?id=38&lang=E&print=true, accessed January 11, 2025 ("INNOTEST")
1025	Galasko, "Biomarkers for Alzheimer's disease – Clinical needs and application", J Alzheimer's Disease 8:339-346 (2005) ("Galasko")
1026	Vandermeeren et al., "Detection of τ Proteins in Normal and Alzheimer's Disease Cerebrospinal Fluid with a Sensitive Sandwich Enzyme-Linked Immunosorbent Assay", J Neurochem. 61:1828-1834 (1993) ("Vandermeeren")
1027	Hulstaert et al. / Innogenetics N.V., "Tau as a Marker for Early CNS Damage", WO00/14546 (March 16, 2000) ("Hulstaert")
1028	Vandermeeren et al., "Monoclonal Antibodies Directed Against the Microtubule-Associated Protein Tau, and Hybridomas Secreting These Antibodies", US Patent No. 5,843,779, issued December 1, 1998 ("Vandermeeren '779")
1029	de Jong, et al., "Current state and future directions of neurochemical biomarkers for Alzheimer's disease" Clin. Chem. Lab. Med. 45: 1421–1434, 1424-25 (2007)
1030	May 29, 2010 Screenshot of "Alzheimer's Disease" webpage of Quanterix downloaded from "Wayback Machine", https://web.archive.org/web/20100529185326/http://www.quanterix.com/applications/ad.html, accessed January 7, 2025 ("PO Website May 29, 2010")
1031	Schraen-Maschke et al., "Tau as a biomarker of neurodegenerative diseases", Biomark Med 2(4): 363-384 (2008) ("Schraen-Maschke")
1032	Blennow et al., CSF markers for incipient Alzheimer's disease", Lancet Neurol 2(10):605-13 (2003) ("Blennow")
1033	Quanterix Single Molecule Arrays for Digital Detection in Complex Samples, IQT Technology Focus Day, March 25, 2010 presentation ("Quanterix Presentation")

Exhibit #	***Description***
1034	October 22, 2013 Screenshot "simoa Assay Kits" webpage of Quanterix downloaded from "Wayback Machine", https://web.archive.org/web/20131022204103/http://quanterix.com/products/assay-kits,, accessed May 27, 2025 (showing Tau Simoa Assay commercially available) ("PO Website Oct 22, 2013")
1035	Nalefski, *et al.,* "Single-Molecule Detection for Femtomolar Quantification of Proteins in Heterogeneous Immunoassays", Clinical Chemistry 52(11): 2172–2175 (1 November 2006), https://doi.org/10.1373/clinchem.2006.072850 and DOI: 10.1373/clinchem.2006.072850
1036	September 9, 2009 Screenshot "Singulex® Assay Menu Erenna® Immunoassay System", Aβ-42 (Amyloid beta protein 42) webpage of Singulex, Inc. downloaded from "Wayback Machine", https://web.archive.org/web/20090912044340/http://www.singulex.com:80/assay_details.html?id=2, accessed April 24, 2025 ("Singulex Website Aug. 12, 2009")
1037	Gavett BE, *et al.*: "Mild traumatic brain injury: a risk factor for neurodegeneration." Alzheimer's Research & Therapy 2010, 2:18, doi:10.1186/alzrt42
1038	Mortimer JA, *et al.*: "Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case–control studies." EURODEM Risk Factors Research Group. Int J Epidemiol 1991, 20:S28-S35.
1039	Quanterix July 30, 2013 Press Release "Quanterix Announces Commercial Availability of its Simoa Single Molecule Array Technology", available at, https://www.quanterix.com/wp-content/uploads/2020/12/Simoa_7-30-13_FINAL-Press-Release.pdf, accessed May 28, 2025 ("Quanterix 7/30/13 Press Release")
1040	Quanterix August 7, 2012 Press Release "Quanterix Issued Key Patent for Simoa™ Technology", available at, https://www.biospace.com/quanterix-issued-key-patent-for-simoa-and-0153-technology, accessed May 28, 2025 ("Quanterix 8/17/12 Press Release")
1041	Quanterix October 16, 2013 Press Release "Quanterix Announces Key Patents Issued for Simoa Platform", available at, https://www.quanterix.com/wp-content/uploads/2020/12/Patents_Release_FINAL.pdf, accessed May 28, 2025 ("Quanterix 10/16/13 Press Release")

Exhibit #	***Description***
1042	Bogoslovsky et al., "Increases of Plasma Levels of Glial Fibrillary Acidic Protein, Tau, and Amyloid β up to 90 Days after Traumatic Brain Injury", J Neurotrauma 34:66–73 (2017) (citation 28 of Ding) ("Bogoslovsky")
1043	Declaration of Ingrid Hsieh-Yee