INTELLECTUAL PROPERTY AND INNOVATION AMERICAN INN OF COURT

Thursday, October 17, 2019

Inn Luncheon Roundtable

CLE Materials

Topic

Athena Diagnostics v. Mayo Collaborative Services and the Future of Patentable Subject Matter

Facilitated By

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Contents

Manual of Patent Examining Procedure 2106: Patent Subject Matter Eligibility

U.S. Patent 7,267,820

Athena Diagnostics v. Mayo Collaborative Services

"Athena v. Mayo: A Splintered Federal Circuit Invites Supreme Court or Congress to Step Up on 101 Chaos"

2106 Patent Subject Matter Eligibility [R-08.2017]

I. TWO CRITERIA FOR SUBJECT MATTER ELIGIBILITY

First, the claimed invention must be to one of the four statutory categories. 35 U.S.C. 101defines the four categories of invention that Congress deemed to be the appropriate subject matter of a patent: processes, machines, manufactures and compositions of matter. The latter three categories define "things" or "products" while the first category defines "actions" (i.e., inventions that consist of a series of steps or acts to be performed). See 35 U.S.C. 100(b)("The term 'process' means process, art, or method, and includes a new use of a known process, machine, manufacture, composition of matter, or material."). See MPEP § 2106.03for detailed information on the four categories.

Second, the claimed invention also must qualify as patent-eligible subject matter, i.e., the claim must not be directed to a judicial exception unless the claim as a whole includes additional limitations amounting to significantly more than the exception. The judicial exceptions (also called "judicially recognized exceptions" or simply "exceptions") are subject matter that the courts have found to be outside of, or exceptions to, the four statutory categories of invention, and are limited to abstract ideas, laws of nature and natural phenomena (including products of nature). *Alice Corp. Pty. Ltd. v. CLS Bank Int'l,* 573 U.S. _, 134 S. Ct. 2347, 2354, 110 USPQ2d 1976, 1980 (2014) (citing *Ass'n for Molecular Pathology v. Myriad Genetics, Inc.,* 569 U.S. _, 133 S. Ct. 2107, 2116, 106 USPQ2d 1972, 1979 (2013). See MPEP § 2106.04for detailed information on the judicial exceptions.

Because abstract ideas, laws of nature, and natural phenomenon "are the basic tools of scientific and technological work", the Supreme Court has expressed concern that monopolizing these tools by granting patent rights may impede innovation rather than promote it. See *Alice Corp.,* 134 S. Ct. at 2354, 110 USPQ2d at 1980; *Mayo Collaborative Servs. v. Prometheus Labs., Inc.,* 566 U.S. 66, 71, 101 USPQ2d 1961, 1965 (2012). However, the Court has also emphasized that an invention is not considered to be ineligible for patenting simply because it involves a judicial exception. *Alice Corp.,* 134 S. Ct. at 2354, 110 USPQ2d at 1980-81 (citing *Diamond v. Diehr,* 450 U.S. 175, 187, 209 USPQ 1, 8 (1981)). See also *Thales Visionix Inc. v. United States,* 850 F.3d. 1343, 1349, 121 USPQ2d 1898, 1902 (Fed. Cir. 2017) ("That a mathematical equation is required to complete the claimed method and system does not doom the claims to abstraction."). Accordingly, the Court has said that an application of an abstract idea, law of nature or natural phenomenon may be eligible for patent protection. *Alice Corp.,* 134 S. Ct. at 2354, 110 USPQ2d at 1980 (citing *Gottschalk v. Benson,* 409 U.S. 63, 67, 175 USPQ 673, 675 (1972)).

The Supreme Court in *Mayo* laid out a framework for determining whether an applicant is seeking to patent a judicial exception itself, or a patent-eligible application of the judicial exception. See *Alice Corp.,* 134 S. Ct. at 2355, 110 USPQ2d at 1981 (citing*Mayo*, 566 U.S. 66, 101 USPQ2d 1961). This framework, which is referred to as the *Mayo* test or the *Alice/Mayo* test, is discussed in further detail in subsection III, below. The first part of the *Mayo* test is to determine whether the claims are directed to an abstract idea, a law of nature or a natural phenomenon (i.e., a judicial exception). *Id.* If the claims are directed to a judicial exception, the second part of the *Mayo* test is to determine whether the claim recites additional elements that amount to significantly more than the judicial exception. *Id.* citing *Mayo,* 566 U.S. at 72-73, 101 USPQ2d at 1966) The Supreme Court has described the second part of the test as the "search for an 'inventive concept'". *Alice Corp.,* 134 S. Ct. at 2355, 110 USPQ2d at 1981 (citing *Mayo*, 566 U.S. at 72-73, 101 USPQ2d at 1966).

The *Alice/Mayo* two-part test is the only test that should be used to evaluate the eligibility of claims under examination. While the machine-or-transformation test is an important clue to eligibility, it should not be used as a separate test for eligibility, but instead should be considered as part of the "significantly more" determination in the *Alice/Mayo* test. *Bilski v. Kappos,* 561 U.S. 593, 605, 95 USPQ2d 1001, 1007 (2010). See MPEP § 2106.05(b)and MPEP § 2106.05(c)for more information about how the machine-or-transformation test fits into the *Alice/Mayo*two-part framework. Likewise, eligibility should not be evaluated based on whether the claim recites a "useful, concrete, and tangible result," *State Street Bank*, 149 F.3d 1368, 1374, 47 USPQ2d 1596, _ (Fed. Cir. 1998) (quoting *In re Alappat,* 33 F.3d 1526, 1544, 31 USPQ2d 1545, _ (Fed. Cir. 1994)), as this test has been superseded. *In re Bilski,* 545 F.3d 943, 959-60, 88 USPQ2d 1385, 1394-95 (Fed. Cir. 2008) (*en banc*), aff'd by *Bilski v. Kappos,* 561 U.S. 593, 95 USPQ2d 1001 (2010). See also *TLI Communications LLC v. AV Automotive LLC,* 823 F.3d 607, 613, 118 USPQ2d 1744, 1748 (Fed. Cir. 2016) ("It is well-settled that mere recitation of concrete, tangible components is insufficient to confer patent eligibility to an otherwise abstract idea"). The programmed computer or "special purpose computer" test of *In re Alappat*, 33 F.3d 1526, 31 USPQ2d 1545 (Fed. Cir. 1994) (*i.e.*, the rationale that an otherwise ineligible algorithm or software could be made patent-eligible by merely adding a generic computer to the claim for the "special purpose" of executing the algorithm or software) was also superseded by the Supreme Court's *Bilski*and *Alice Corp.* decisions. *Eon Corp. IP Holdings LLC v. AT&T Mobility LLC,* 785 F.3d 616, 623, 114 USPQ2d 1711, 1715 (Fed. Cir. 2015) ("[W]e note that *Alappat* has been superseded by *Bilski,* 561 U.S. at 605–06, and *Alice Corp. v. CLS Bank Int'l,* 134 S. Ct. 2347 (2014)"); *Intellectual Ventures I LLC v. Capital One Bank (USA), N.A.,* 792 F.3d 1363, 1366, 115 USPQ2d 1636, 1639 (Fed. Cir. 2015) ("An abstract idea does not become nonabstract by limiting the invention to a particular field of use or technological environment, such as the Internet [or] a computer"). Lastly, eligibility should not be evaluated based on whether the claimed invention has utility, because "[u]tility is not the test for patent-eligible subject matter." *Genetic Techs. Ltd. v. Merial LLC,*818 F.3d 1369, 1380, 118 USPQ2d 1541, 1548 (Fed. Cir. 2016).

Examiners are reminded that 35 U.S.C. 101is not the sole tool for determining patentability; 35 U.S.C. 112, 35 U.S.C. 102, and 35 U.S.C. 103will provide additional tools for ensuring that the claim meets the conditions for patentability. As the Supreme Court made clear in *Bilski,* 561 U.S. at 602, 95 USPQ2d at 1006:

> The § 101patent-eligibility inquiry is only a threshold test. Even if an invention qualifies as a process, machine, manufacture, or composition of matter, in order to receive the Patent Act's protection the claimed invention must also satisfy "the conditions

and requirements of this title." § 101. Those requirements include that the invention be novel, see § 102, nonobvious, see § 103, and fully and particularly described, see § 112.

II. ESTABLISH BROADEST REASONABLE INTERPRETATION OF CLAIM AS A WHOLE

It is essential that the broadest reasonable interpretation (BRI) of the claim be established prior to examining a claim for eligibility. The BRI sets the boundaries of the coverage sought by the claim and will influence whether the claim seeks to cover subject matter that is beyond the four statutory categories or encompasses subject matter that falls within the exceptions. Evaluating eligibility based on the BRI also ensures that patent eligibility under 35 U.S.C. 101does not depend simply on the draftsman's art. *Alice,* 134 S. Ct. at 2359, 2360, 110 USPQ2d at 1984, 1985 (citing *Parker v. Flook,* 437 U.S. 584, 593, 198 USPQ 193, 198 (1978) and *Mayo,* 566 U.S. at 72, 101 USPQ2d at 1966). See MPEP § 2111for more information about determining the BRI.

Claim interpretation affects the evaluation of both criteria for eligibility. For example, in *Mentor Graphics v. EVE-USA, Inc.,* 851 F.3d 1275, 112 USPQ2d 1120 (Fed. Cir. 2017), claim interpretation was crucial to the court's determination that claims to a "machine-readable medium" were not to a statutory category. In *Mentor Graphics,* the court interpreted the claims in light of the specification, which expressly defined the medium as encompassing "any data storage device" including random-access memory and carrier waves. Although random-access memory and magnetic tape are statutory media, carrier waves are not because they are signals similar to the transitory, propagating signals held to be non-statutory in *Nuijten*. 851 F.3d at 1294, 112 USPQ2d at 1133 (citing *In re Nuijten,* 500 F.3d 1346, 84 USPQ2d 1495 (Fed. Cir. 2007)). Accordingly, because the BRI of the claims covered both subject matter that falls within a statutory category (the random-access memory), as well as subject matter that does not (the carrier waves), the claims as a whole were not to a statutory category and thus failed the first criterion for eligibility.

With regard to the second criterion for eligibility, the *Alice/Mayo* test, claim interpretation can affect the first part of the test (whether the claims are directed to a judicial exception). For example, the patentee in *Synopsys* argued that the claimed methods of logic circuit design were intended to be used in conjunction with computer-based design tools, and were thus not mental processes. *Synopsys, Inc. v. Mentor Graphics Corp.,* 839 F.3d 1138, 1147-49, 120 USPQ2d 1473, 1480-81 (Fed. Cir. 2016). The court disagreed, because it interpreted the claims as encompassing nothing other than pure mental steps (and thus an abstract idea) because the claims did not include any limitations requiring computer implementation. In contrast, the patentee in *Enfish*argued that its claimed self-referential table for a computer database was an improvement in an existing technology and thus not directed to an abstract idea. *Enfish, LLC v. Microsoft Corp.,* 822 F.3d 1327, 1336-37, 118 USPQ2d 1684, 1689-90 (Fed. Cir. 2016). The court agreed with the patentee, based on its interpretation of the claimed "means for configuring" under 35 U.S.C. 112(f)as requiring a four-step algorithm that achieved the improvements, as opposed to merely any form of storing tabular data. See also *McRO, Inc. v. Bandai Namco Games America, Inc.* 837 F.3d 1299, 1314, 120 USPQ2d 1091, 1102 (Fed. Cir. 2016) (the claim's construction incorporated rules of a particular type that improved an existing technological process). Claim interpretation can also affect the second part of the *Alice/Mayo* test (whether the claim recites additional elements that amount to significantly more than the judicial exception). For example, in *Amdocs (Israel) Ltd. v. Openet Telecom, Inc.,* where the court relied on the construction of the term "enhance" (to require application of a number of field enhancements in a distributed fashion) to determine that the claim entails an unconventional technical solution to a technological problem. 841 F.3d 1288, 1300-01, 120 USPQ2d 1527, 1537 (Fed. Cir. 2016).

III. SUMMARY OF ANALYSIS AND FLOWCHART

Examiners should determine whether a claim satisfies the criteria for subject matter eligibility by evaluating the claim in accordance with the following flowchart. The flowchart illustrates the steps of the subject matter eligibility analysis for products and processes that are to be used during examination for evaluating whether a claim is drawn to patent-eligible subject matter. It is recognized that under the controlling legal precedent there may be variations in the precise contours of the analysis for subject matter eligibility that will still achieve the same end result. The analysis set forth herein promotes examination efficiency and consistency across all technologies.

As shown in the flowchart, Step 1 relates to the statutory categories and ensures that the first criterion is met by confirming that the claim falls within one of the four statutory categories of invention. See MPEP § 2106.03for more information on Step 1. Step 2, which is the Supreme Court's *Alice/Mayo* test, is a two-part test to identify claims that are directed to a judicial exception (Step 2A) and to then evaluate what more such claims recite to provide an inventive concept (Step 2B) (also called a practical application) to the judicial exception. See MPEP § 2106.04for more information on Step 2A, and MPEP § 2106.05for more information on Step 2B.

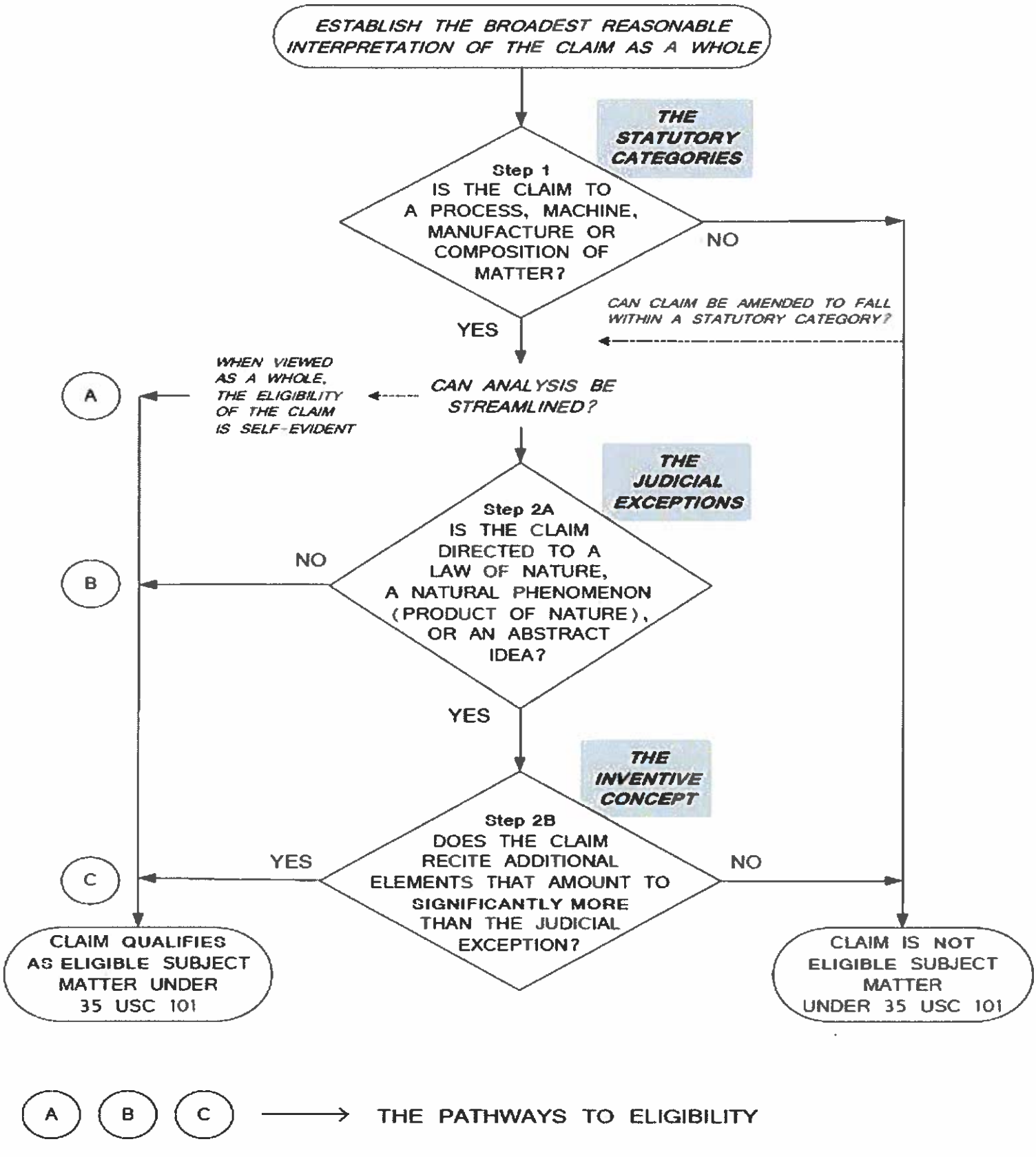
The flowchart also shows three pathways (A, B, and C) to eligibility:

- Pathway A: Claims taken as a whole that fall within a statutory category (Step 1: YES) and, which may or may not recite a judicial exception, but whose eligibility is self-evident can be found eligible at Pathway A using a streamlined analysis. See MPEP § 2106.06for more information on this pathway and on self-evident eligibility.
- Pathway B: Claims taken as a whole that fall within a statutory category (Step 1: YES) and are not directed to a judicial exception (Step 2A: NO) are eligible at Pathway B. These claims do not need to go to Step 2B. See MPEP § 2106.04for more information about this pathway and Step 2A.
- Pathway C: Claims taken as a whole that fall within a statutory category (Step 1: YES), are directed to a judicial exception (Step 2A: YES), and recite additional elements either individually or in an ordered combination that amount to significantly more than the judicial exception (Step 2B: YES) are eligible at Pathway C. See MPEP § 2106.05for more information about this pathway and Step 2B.

Claims that could have been found eligible at Pathway A (streamlined analysis), but are subjected to further analysis at Steps 2A or Step 2B, will ultimately be found eligible at Pathways B or C. Thus, if the examiner is uncertain about whether a streamlined analysis is appropriate, the examiner is encouraged to conduct a full eligibility analysis. However, if the claim is not found eligible at any of Pathways A, B or C, the claim is patent ineligible and should be rejected under **35 U.S.C. 101**.

Regardless of whether a rejection under **35 U.S.C. 101**is made, a complete examination should be made for every claim under each of the other patentability requirements: **35 U.S.C. 102**, **103**, **112**, and **101**(utility, inventorship and double patenting) and non-statutory double patenting. **MPEP § 2103**.

SUBJECT MATTER ELIGIBILITY TEST FOR PRODUCTS AND PROCESSES



A B C ⟶ THE PATHWAYS TO ELIGIBILITY

US007267820B2

(12) **United States Patent**
Vincent et al.

(10) **Patent No.: US 7,267,820 B2**
(45) **Date of Patent: Sep. 11, 2007**

(54) **NEUROTRANSMISSION DISORDERS**

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(73) Assignees: **Isis Innovation Limited**, Oxford (GB); **Max-Planck Gesellschaft zur Foerderung der Wissenschaften e.V.**, Munich (DE)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 506 days.

(21) Appl. No.: **10/311,575**

(22) PCT Filed: **Jun. 15, 2001**

(86) PCT No.: **PCT/GB01/02661**

§ 371 (c)(1),
(2), (4) Date: **Jun. 6, 2003**

(87) PCT Pub. No.: **WO01/96601**

PCT Pub. Date: **Dec. 20, 2001**

(65) **Prior Publication Data**

US 2004/0082010 A1 Apr. 29, 2004

(30) **Foreign Application Priority Data**

Jun. 16, 2000 (GB) 0014878.3

(51) **Int. Cl.**
A61K 39/395 (2006.01)
A61K 39/00 (2006.01)
A61K 38/00 (2006.01)
C07K 14/00 (2006.01)

(52) **U.S. Cl.** **424/130.1**; 424/184.1; 424/178.1; 514/2; 530/350

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(Continued)

Primary Examiner—Eileen O'Hara
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(74) *Attorney, Agent, or Firm*—Hamilton, Brook, Smith & Reynolds, P.C.

(57) **ABSTRACT**

There is disclosed a method for diagnosing neurotransmission or developmental disorders in a mammal comprising the step of detecting in a bodily fluid of said mammal autoantibodies to an epitope of the muscle specific tyrosine kinase (MuSK). One such method comprises a) contacting said bodily fluid with said MuSK or an antigenic determinant thereof; and b) detecting any antibody-antigen complexes formed between said receptor tyrosine kinase or an antigenic fragment thereof and antibodies present in said bodily fluid, wherein the presence of said complexes is indicative of said mammal suffering from said neurotransmission or developmental disorders. Also disclosed are kits for use in the diagnosis of neurotransmission and subsequent developmental disorders.

12 Claims, 6 Drawing Sheets

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FIG. 1.

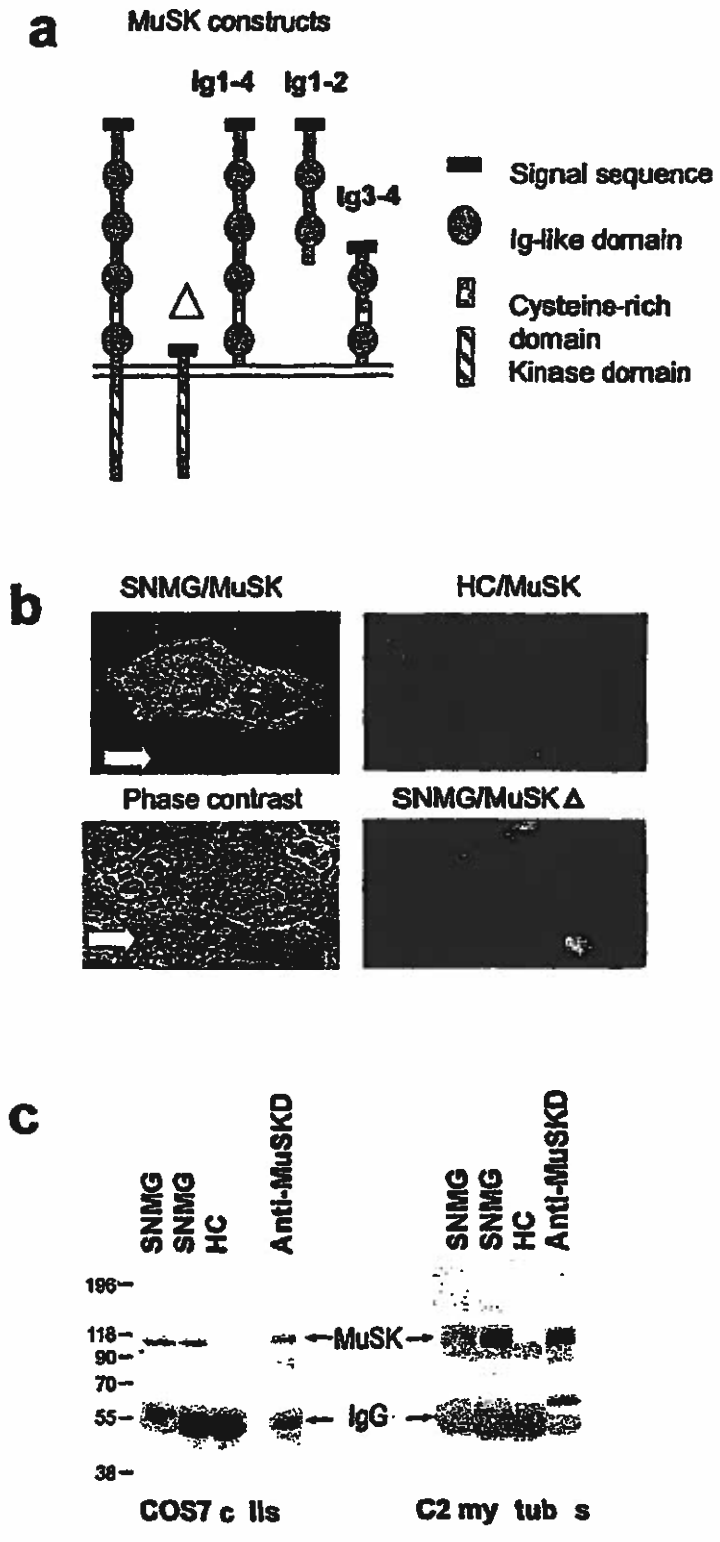
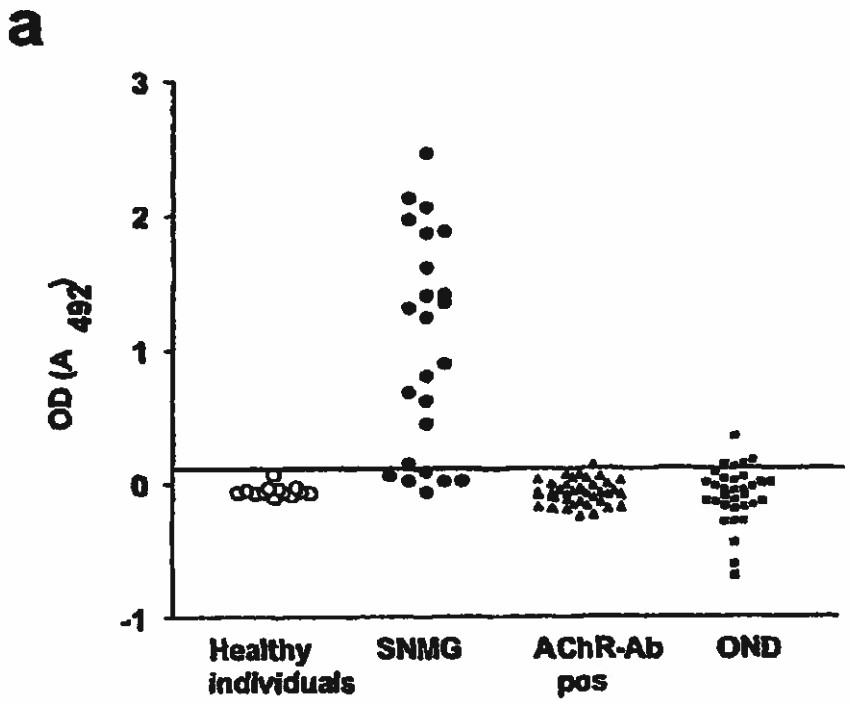

a
MuSK constructs
Ig1-4
Ig1-2
Ig3-4
Signal sequence
Ig-like domain
Cysteine-rich domain
Kinase domain
b
SNMG/MuSK
HC/MuSK
Phase contrast
SNMG/MuSKΔ
c
SNMG
SNMG
HC
Anti-MuSKD
SNMG
SNMG
HC
Anti-MuSKD
196
118
90
70
55
38
MuSK
IgG
COS7 c lls
C2 my tub s

FIG. 2.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Healthy individuals
SNMG
AChR-Ab pos
OND

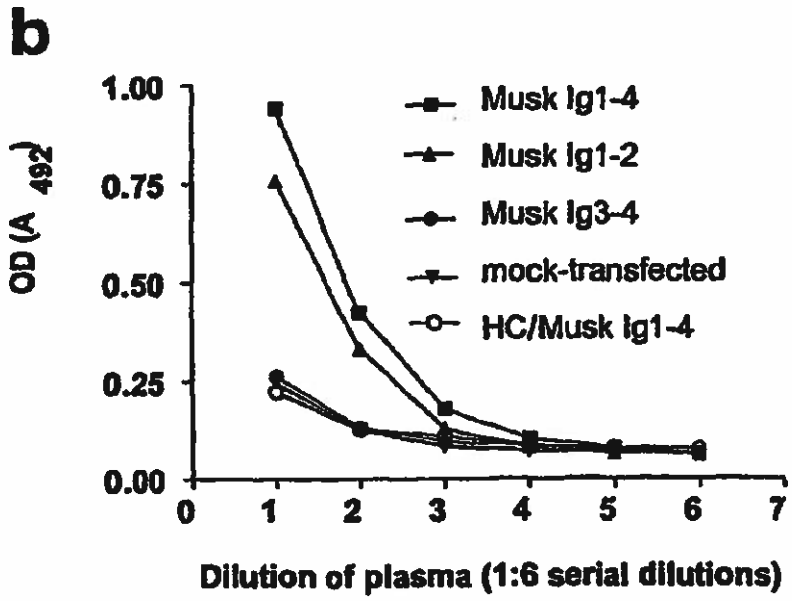

b
1.00
0.75
0.50
0.25
0.00
OD (A 492)
0
1
2
3
4
5
6
7
Musk Ig1-4
Musk Ig1-2
Musk Ig3-4
mock-transfected
HC/Musk Ig1-4
Dilution of plasma (1:6 serial dilutions)

FIG. 3.

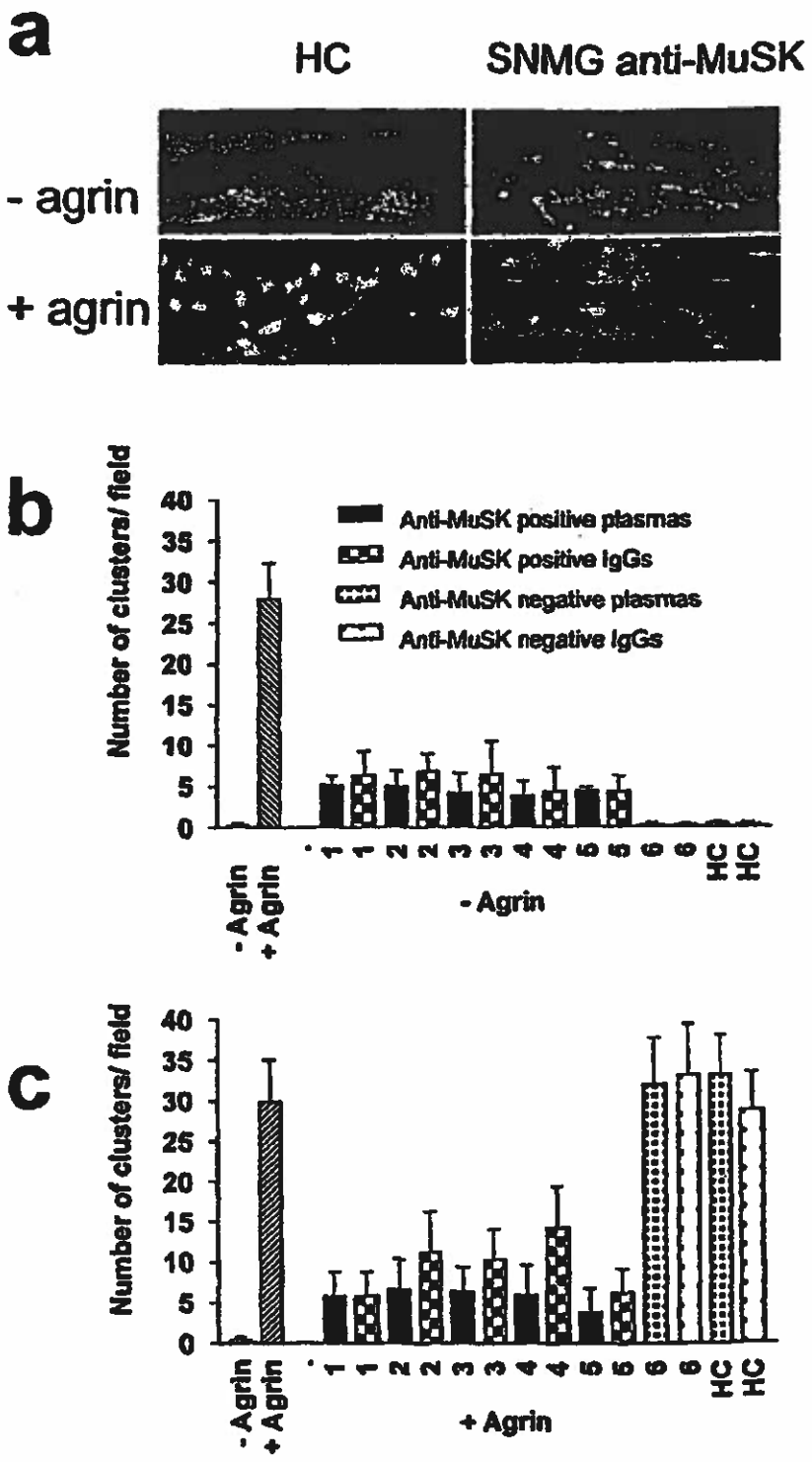

a
HC
SNMG anti-MuSK
- agrin
+ agrin
b
Number of clusters/ field
Anti-MuSK positive plasmas
Anti-MuSK positive IgGs
Anti-MuSK negative plasmas
Anti-MuSK negative IgGs
- Agrin
+ Agrin
- Agrin
c
Number of clusters/ field
- Agrin
+ Agrin
+ Agrin

FIG. 4.

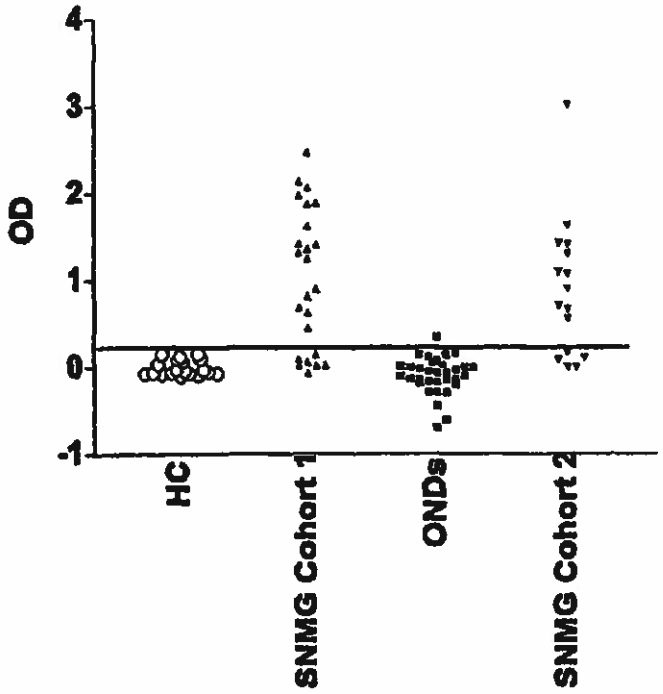

OD
4
3
2
1
0
-1
HC
SNMG Cohort 1
ONDs
SNMG Cohort 2

FIG. 5.

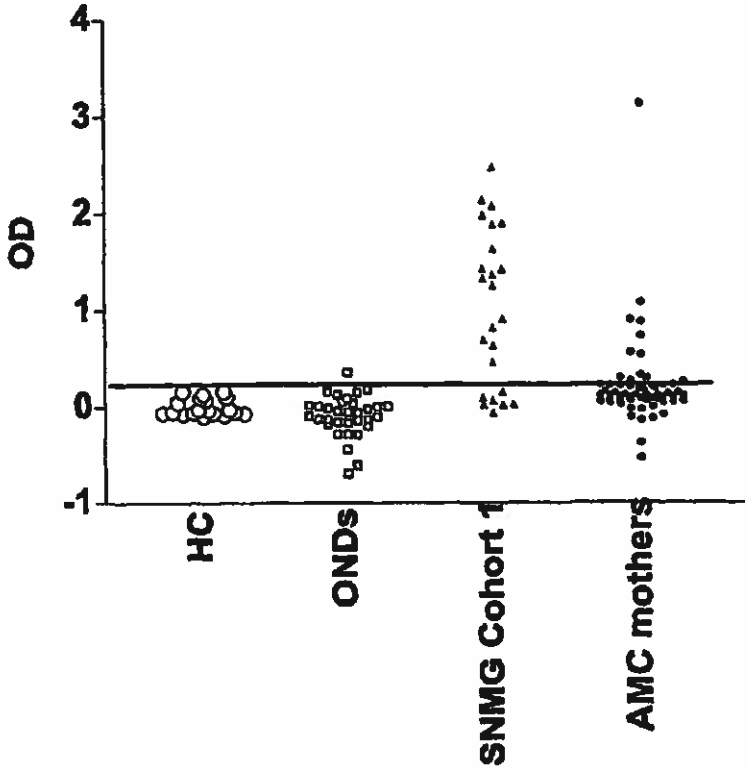

OD
4
3
2
1
0
-1
HC
ONDs
SNMG Cohort 1
AMC mothers

FIG. 6.

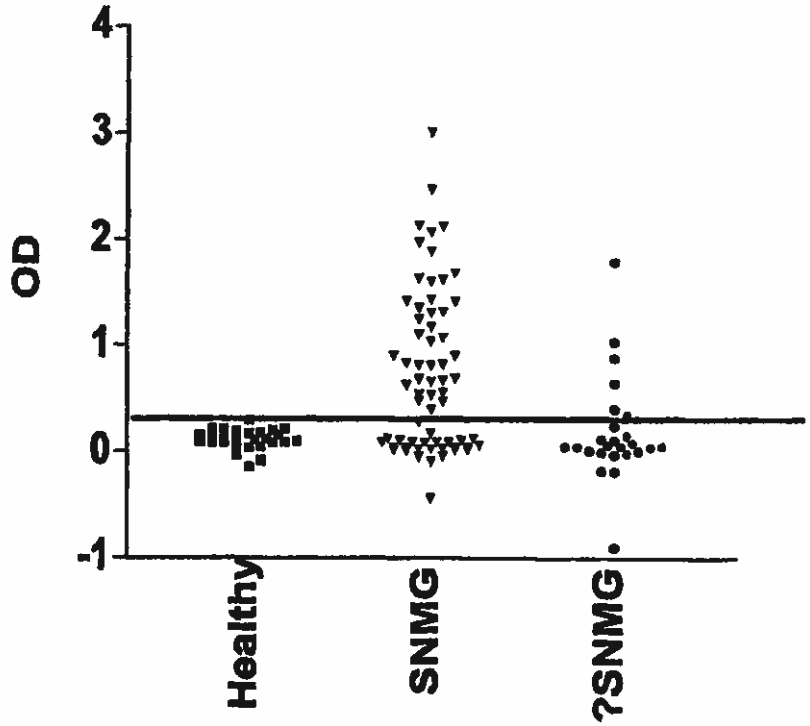

OD
4
3
2
1
0
-1
Healthy
SNMG
?SNMG

FIG. 7.

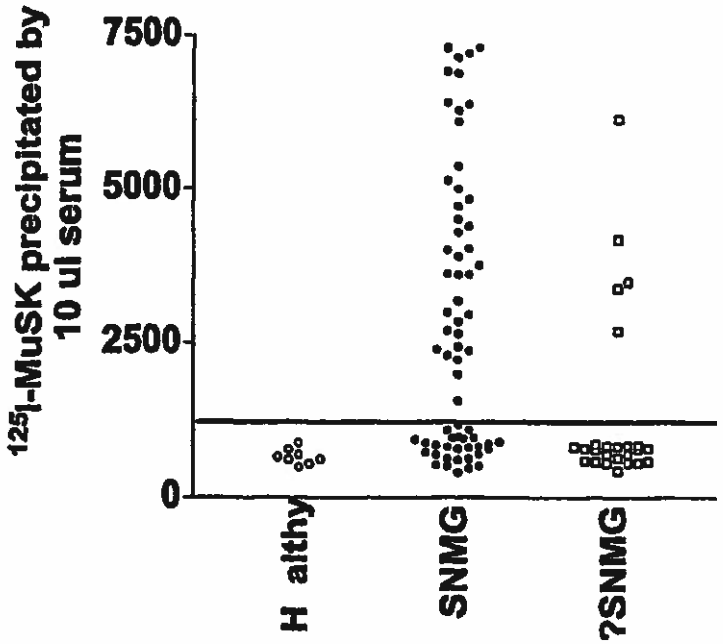
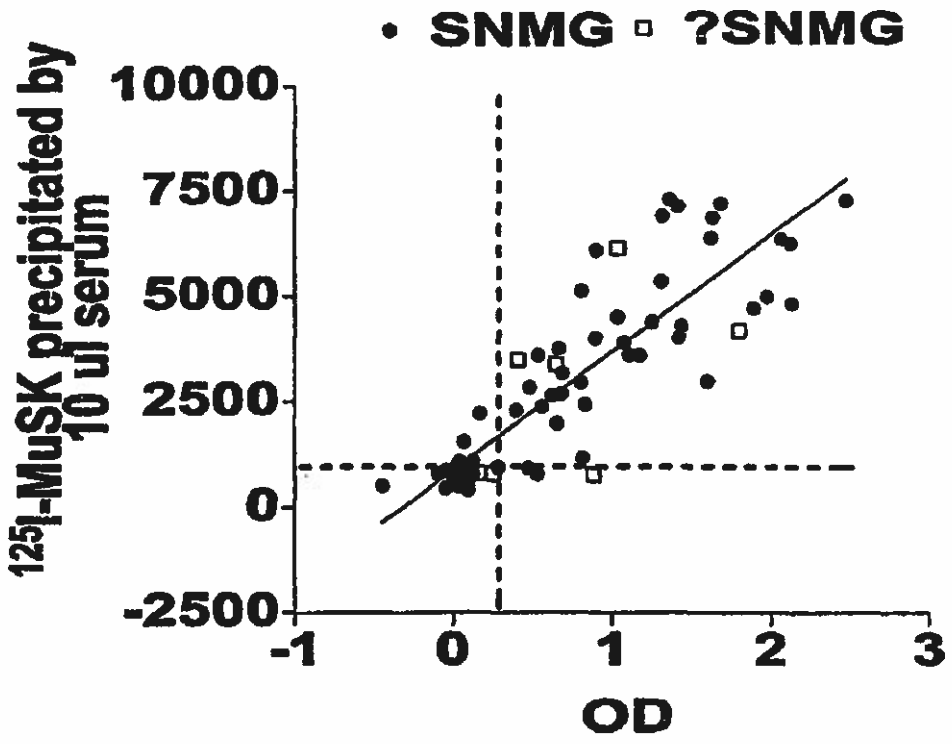

125I-MuSK precipitated by 10 ul serum
7500
5000
2500
0
H althy
SNMG
?SNMG

FIG. 8.


• SNMG ▫ ?SNMG
^{125}I-MuSK precipitated by 10 ul serum
10000
7500
5000
2500
0
-2500
-1
0
1
2
3
OD

NEUROTRANSMISSION DISORDERS

RELATED APPLICATIONS

This application is a national stage filing under 35 U.S.C. § 371 of PCT International application PCT/GB01/02661, filed Jun. 15, 2001, which was published under PCT Article 21(2) in English.

The present invention is concerned with neurotransmission disorders and, in particular, with a method of diagnosing such disorders in mammals. Also provided by the present invention are kits for use in said diagnosis.

Myasthenia gravis (MG) is a chronic autoimmune disorder of neuromuscular transmission resulting in muscle weakness. The key feature of weakness due to MG is its variability. Patients generally experience a waning of strength throughout the day with a tendency to fatigue later in the day or even towards the end of a particular task. A symptom of MG is often ocular weakness, causing ptosis (drooping eyelids) and/or diplopia (double vision). Other symptoms include leg weakness, dysphagia and slurred or nasal speech. Symptoms of weakness tend to worsen with various stressors, such as, exertion, heat and infection.

In 1960 it was discovered that MG was caused by antibodies against the acetyl choline receptor (AChR) and that it is therefore autoimmune in origin. Today MG is one of the most characterised of neurological disorders which has consequently lead to treatments which vastly improve the length and quality of life of myasthenics. Approximately 10 people in every million of a population contract this disease in one year. There is no racial predominance and 75% of MG patients less than 40 years of age are female and 60% of those older than 40 years are male.

Approximately 80% of patients with MG possess within their plasma autoantibodies that are immunoprecitipatable with radiolabelled AChR. The remaining 20% of MG patients do not, however, exhibit such antibodies in their plasma but do have similar symptoms and respond to the same therapies such as plasma exchange and immunosuppression. Accordingly, it has not been established whether these patients have the same or a distinct and separate MG condition(3,4). Autoantibodies are naturally occurring antibodies directed to an antigen which an individual's immune response recognises as foreign even though that antigen actually originated in the individual. They may be present in the circulatory system as circulating free antibodies or in the form of circulating immune complexes bound to their target depending on the nature of the antigen concerned.

Human plasma from patients who were anti-AChR autoantibodies negative (AAAN or previously known as sero-negative MG), were investigated for alternative autoantibodies and one candidate autoantibody was that one for the MuSK protein.

The present inventors surprisingly found that many of the 20% of MG patients which do not exhibit any autoantibodies to AChR, instead have IgG antibodies directed against the extracellular N-terminal domains of MuSK, a receptor tyrosine kinase located on the cell surface of neuromuscular junctions, indicating that they are afflicted with a form of MG which has a different etiology from MG characterised by circulating autoantibodies to AChR.

The MuSK protein has been sequenced and the protein characterised recently by Valenzuela et al (International patent application number PCT/US96/20696, published as WO97/21811). It is a receptor tyrosine kinase (RTK) located on the cell surface of muscle cells at the neuromuscular junction. Ligands bind to RTKs at the binding site on the extracellular side of the receptor, which induces transmission of a signal cascade to intracellular target proteins. RTKs are classified according to their function and members of these families share high homology in their amino acid sequence as well as functionality.

At the neuromuscular junction (NMJ) where the motor nerve axon dendrites meet the muscle cell basal membrane, important physiological signals are exchanged between these adjacent cells. An example of this is the chemical transmitter acetyl choline which passes through the synaptic cleft from the nerve cell, and is then rapidly and specifically bound by the AChR at the muscle cell wall. This in turn begins a cascade of events which ultimately leads to contraction of the muscle cells.

The post synaptic structure at the muscle cell wall is termed the motor endplate which is densely packed with protein and lipid, thereby giving an electron dense appearance when observed by electron microscopy. The muscle AChRs are present here, and it is believed that signalling gives rise to concentrations of proteins there by two mechanisms; one is altered distribution of pre-existing membrane proteins and the other is by induction of localised transcription of specific genes only by subsynaptic nuclei underlying the NMJ.

Development of the neuromuscular junction is initiated through activation of MuSK. Agrin isoforms, released from the motorneuron, trigger MuSK and muscle acetylcholine receptor (AChR) phosphorylation resulting in clustering of AChRs and other proteins of the postsynaptic apparatus(1). Agrin's ability to cause AChR clustering in cultured myotubes has been shown to be inhibited by anti agrin antibodies. It is currently accepted that agrin does not bind directly to MUSK, but via a hypothetical agrin-binding component termed Myotubule Associated Specificity Component (MASC) (1,11). No disease associated with either MuSK, MASC, or agrins has been reported and their roles in adult muscle have not yet been elucidated.

It has already been shown that anti AChR autoantibody negative MG is caused by humoral IgG antibodies: it can be successfully treated by plasma exchange and other immune therapies(5); transient neonatal MG was reported in the newborn infant of one of the patients with anti-MuSK antibodies(17); and injection of immunoglobulin or IgG preparations into mice caused defects in neuromuscular transmission (5).

The present inventors have therefore now shown that anti-MuSK antibodies have functional effects on agrin-induced AChR clustering in vitro, and direct interference with this agrin/MuSK/AChR pathway may be an important disease mechanism in vivo. MuSK is a relatively new member of the receptor tyrosine kinase (RTK) family. With very few exceptions (for example, see 18), autoantibodies to RTKs have not been implicated in human disorders but the combination of large extracellular domains and functional activities make them attractive potential antigens in other autoimmune conditions. Other members of the RTK family are mutated in inherited diseases, and somatic mutations have been found in various tumors (19). MuSK may prove to be involved in congenital as well as acquired muscle disorders.

Therefore, there is provided by a first aspect of the present invention a method of diagnosing neurotransmission disorders in a mammal comprising the step of detecting in a bodily fluid of said mammal autoantibodies to an epitope of the muscle specific tyrosine kinase, MuSK.

More specifically the neurotransmission disorder will preferably be *Myasthenia gravis* and more particularly a

subclass or subtype of MG which is generally found in patients who do not exhibit the ability to immunprecipitate radiolabelled AChR with their bodily fluids.

This aspect of the invention is particularly advantageous because the identification of this new subclass or subtype of MG patients will allow for more accurate and speedy diagnosis of individuals by medical practitioners. The method according to this aspect of the invention will allow for detection of neurotransmission abnormalities that are either congenital or acquired, for example, postnatally or prenatally from transmission from the mother to the foetus. As set out in more detail in the example provided, some mothers of babies with developmental disorders, such as paralysis and fixed joints were identified as having antibodies to MuSK, which were transferred placentally.

Until now, MuSK has been studied primarily in NMJ development. The presence of antibodies to the extracellular domain of MuSK in an acquired disorder implies that MuSK is functional at the adult NMJ, and implicates MuSK as a novel target for pathogenic autoantibodies causing *Myasthenia gravis*. The isolation and purification of this anti-MUSK autoantibody will give rise to a useful product which may be exploitable as an indicator of neurotransmission diseases.

Preferably, the method according to the first aspect of the invention, comprises the steps of a) contacting said bodily fluid with said MUSK or an antigenic determinant thereof; and b) detecting any antibody-antigen complexes formed between said MuSK or an antigenic fragment thereof and antibodies present in said bodily fluid, wherein the presence of said complexes is indicative of said mammal suffering from said neurotransmission disorders.

The actual steps of detecting autoantibodies in a sample of bodily fluids may be performed in accordance with immunological assay techniques known per se in the art. Examples of suitable techniques include ELISA, radioimmunoassays and the like. In general terms, such assays use an antigen which may be immobilised on a solid support. A sample to be tested is brought into contact with the antigen and if autoantibodies specific to the protein are present in a sample they will immunologically react with the antigen to form autoantibody-antigen complexes which may then be detected or quantitatively measured. Detection of autoantibody-antigen complexes is preferably carried out using a secondary anti-human immunoglobulin antibody, typically anti-IgG or anti-human IgM, which recognizes general features common to all human IgGs or IgMs, respectively. The secondary antibody is usually conjugated to an enzyme such as, for example, horseradish peroxidase (HRP) so that detecting of autoantibody/antigen/secondary antibody complexes is achieved by addition of an enzyme substrate and subsequent calorimetric, chemiluminescent or fluorescent detection of the enzymatic reaction products.

Thus, in one embodiment the antibody/antigen complex may be detected by a further antibody, such as an anti-IgG antibody. Complexes may alternatively be viewed by microscopy. Other labels or reporter molecules which may be used in a method according to the invention. Preferably, said reporter molecule or label includes any of a heavy metal, a fluorescent or luminescent molecule, radioactive or enzymatic tag. Preferably, the label or reporter molecule is such that the intensity of the signal from the anti-human IgG antibody is indicative of the relative amount of the anti-MuSK autoantibody in the bodily fluid when compared to a positive and negative control reading.

An alternative method of detecting autoantibodies for MuSK or an epitope thereof relies upon the binding of a MuSK or its epitope, together with a revealing label, to the autoantibodies in the serum or bodily fluid. This method comprises contacting MuSK or an epitope or antigenic determinant thereof having a suitable label thereon, with said bodily fluid, immunoprecipitating any antibodies from said bodily fluid and monitoring for said label on any of said antibodies, wherein the presence of said label is indicative of said mammal suffering from said neurotransmission or developmental disorder. Preferably, the label is a radioactive label which may be ^{125}I, or the like. Iodination and immunoprecipitation are standard techniques in the art, the details of which may be found in references (4 and 6).

In a further aspect of the invention, there is provided an assay kit for diagnosing neurotransmission disorders in mammals comprising an epitope of muscle specific tyrosine kinase (MuSK) and means for contacting said MuSK with a bodily fluid from a mammal. Thus advantageously, an assay system for detecting neurotransmission disorders, and particularly *Myasthenia gravis* in patients who are anti-AChR autoantibody negative (AAAN) is provided. Prior to the present invention there was no basis for providing an immediate clinical diagnosis for such patients.

Also provided by the invention is an isolated or purified autoantibody specific for MuSK. Such an antibody can be detected in bodily fluids of mammals and isolated or purified therefrom using techniques which would be known to the skilled practitioner, such as, immunoabsorption, or immunoaffinity chromatography or high pressure chromatography.

In a further aspect the invention also comprises an isolated or purified antibody specific for an anti-MuSK autoantibody from bodily fluid of a mammal. Such a purified or isolated antibody which is specific for anti-MuSK autoantibody may advantageously be used as a medicament, or in the preparation of a medicament for treating neurotransmission disorders in a mammal, and preferably a human suffering from *Myasthenia gravis*. Such an antibody may also be included in a pharmaceutical composition together with a pharmaceutically acceptable carrier, excipient or diluent therefor. Antibodies, polyclonal or monoclonal may be prepared using techniques which are known in the art. For example, the technique described by Kohler & Milstein (1975, Nature 256:495-497) for developing hybridomas capable of producing monoclonal antibodies may be used. Monoclonal antibodies for therapeutic use may be human monoclonal antibodies or chimeric human-mouse monoclonal antibodies. Chimeric antibody molecules may be prepared containing a mouse antigen binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. USA 81:6581, Takeda et al., 1985, Nature 314: 452). For production of antibody various host animals can be immunized by injection with anti-MuSK autoantibody, or a fragment or derivative thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronicpolyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*.

The present invention includes not only complete antibody molecules but fragments thereof. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques, for example, such fragments include but are not limited to the $F(ab')_2$ fragment which can be

produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

The antibody which is specific for anti-MuSK autoantibodies may also, advantageously, be used in a diagnostic kit for detecting neurotransmission disorders, such as *Myasthenia gravis*. As aforementioned any protein which binds to the autoantibody may also be used such as an epitope or fragment of the MuSK protein itself. Such a kit comprises an isolated or purified antibody specific for anti-MuSK autoantibody according to the invention and means for contacting said antibody with a bodily fluid of a said mammal.

In accordance with the present invention a bodily fluid should be taken to mean plasma, serum, whole blood, urine, sweat, lymph, faeces, cerebrospinal fluid or nipple aspirate. In general, however, the methods of the invention will be performed on samples of serum or plasma.

In the pharmaceutical composition of the invention, preferred compositions include pharmaceutically acceptable carriers including, for example, non-toxic salts, sterile water or the like. A suitable buffer may also be present allowing the compositions to be lyophilized and stored in sterile conditions prior to reconstitution by the addition of sterile water for subsequent administration. The carrier can also contain other pharmaceutically acceptable excipients for modifying other conditions such as pH, osmolarity, viscosity, sterility, lipophilicity, solubility or the like. Pharmaceutical compositions which permit sustained or delayed release following administration may also be used.

The antibody or the MuSK protein or fragment thereof or the pharmaceutical composition of the invention may be administered orally. In this embodiment the antibody, MuSK or its eptopic fragment, or pharmaceutical composition of the invention may be encapsulated and/or combined with suitable carriers in solid dosage forms which would be well known to those of skill in the art.

Furthermore, as would be appreciated by the skilled practitioner, the specific dosage regime may be calculated according to the body surface area of the patient or the volume of body space to be occupied, dependent on the particular route of administration to be used. The amount of the composition actually administered will, however, be determined by a medical practitioner based on the circumstances pertaining to the disorder to be treated, such as the severity of the symptoms, the age, weight and response of the individual.

In a further aspect, the present invention comprises a method of treating a patient suffering from a neurotransmission disorder such as *Myasthenia gravis* comprising administering to said patient an effective amount of an antibody according to the invention or a MuSK protein or an epitope thereof.

In an even further aspect, the invention comprises a method for making a pharmaceutical formulation for the treatment of neurotransmission disorders, comprising the steps of isolating or purifying an antibody or MuSK protein or fragment thereof according to the invention, manufacturing bulk quantities of said antibody and formulating the antibody in a compound including a pharmaceutically acceptable carrier, diluent or excipient therefor.

In an even further aspect, the invention comprises a method of identifying compounds capable of alleviating or treating neurotransmission disorders, comprising the steps of contacting a candidate compound in the presence of MuSK or an epitope thereof and an antibody capable of binding MuSK, wherein a compound that prevents binding of said antibody to MuSK or an epitope thereof is a candidate for treating neurotransmission disorders. Such compounds may also be used in treating neurotransmission or developmental disorders or in the manufacture of a medicament for treating such disorders. The compounds identified may also, as would be appreciated by those of skill in the art, serve as lead compounds for the development of analogue compounds. The analogues should have a stabilized electronic configuration and molecular conformation that allows key functional groups to be presented to the polypeptides of the invention in substantially the same way as the lead compound. In particular, the analogue compounds have spatial electronic properties which are comparable to the binding region, but can be smaller molecules than the lead compound, frequently having a molecular weight below about 2 kD and preferably below about 1 kD. Identification of analogue compounds can be performed through use of techniques such as self-consistent field (SCF) analysis, configuration interaction (CI) analysis, and normal mode dynamics analysis. Computer programs for implementing these techniques are available; e.g., Rein, Computer-Assisted Modelling of Receptor-Ligand Interactions (Alan Liss, New York, 1989). Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, N.Y., USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art; see also supra. Furthermore, peptidomimetics and/or computer aided design of appropriate derivatives and analogues can be used.

The present invention may be more clearly understood with reference to the following examples and accompanying Figures wherein:

FIG. **1**: is an illustration of the results obtained using antibodies from AAAN patients reacting with the extracellular domain of MuSK. Samples from AAAN patients are indicated as SNMG (sero-negative MG) as it was previously known. a, The MuSK constructs used are shown in FIG. **1***a*. b, AAAN plasmas bound to COS-cells expressing full length MuSK (AAAN/MuSK). MuSK immunoreactivity appeared as a speckled pattern, similar to that seen previously with rabbit anti-MuSK antibodies(13). Non-transfected cells in the same field, demonstrated below by phase contrast microscopy, (arrows), showed non-specific binding only. There was no specific binding of AAAN plasmas to cells expressing MuSK lacking the extracellular domains (MuSK D) or binding of healthy control plasma (HC/MuSK). c, Two AAAN plasmas, but not a healthy control plasma, immunoprecipitated MuSK from detergent extracts of COS-cells expressing MuSK, and C2C12 myotubes. MuSK was identified by binding of an affinity-purified rabbit anti-MuSK. It appears as a 110 kD band from COS-cells and as several bands representing different MuSK splice variants in the C2C12 cells.

FIG. **2**: is an illustration of results obtained by using IgG antibodies to the extracellular domains of MuSK in seronegative MG measured by ELISA. a, Anti-MuSK antibodies were found in 17/24 AAAN patients compared with 13 controls. Negative or borderline values only were found in 39 anti-AChR positive MG patients. Non-specific binding of IgG to the plates has been subtracted. b, Titration of one AAAN plasma against different domains of MuSK. The antibodies bound strongly to MuSK constructs expressing

the distal immunoglobulin like domains. Ig1-4 and Ig1-2 (see FIG 1*a*), but not to the Ig3-4 membrane-proximal domains.

FIG. **3**: is an illustration of the results that show that AAAN antibodies induce AChR clusters but inhibit agrin-induced AChR clustering. a, In the absence of agrin, a moderate number of AChR clusters (as demonstrated by rhodamine-a-bungarotoxin fluorescence) were induced in the presence of AAAN plasma compared to that in control plasma (HC). Agrin-induced clusters were found in the presence of healthy control plasma but were inhibited in the presence of AAAN plasma. b,c, The AChR clusters without (b) or with (c) added agrin in plasma and IgG treated cultures. AAAN samples are labelled 1-6. Only the anti-MuSK positive plasmas and IgG preparations affected AChR clusters.

FIG. **4** is an illustration of the results obtained from further tests to confirm the specificity of the test for *Myasthenia gravis* set out in the examples provided.

FIG. **5** is an illustration of the results obtained from a test to detect MuSK antibodies in mothers of babies with development defects.

FIG. **6** is an illustration of the results obtained using an ELISA assay to detect MuSK antibodies in sera sent for analysis.

FIG. **7** is an illustration of the results obtained using an immunoprecipitation assay to detect MuSK antibodies in the sera of FIG. **6**.

FIG. **8** is correlation of the results of ELISA and immunoprecipitation assays of FIGS. **6** and **7** for detection of MuSK antibodies.

EXAMPLE

Patient Identification

Samples were obtained from 24 patients (18F, 6 M) with moderate or severe generalised MG, diagnosed by clinical electrophysiology, but in whom the standard radioimmunoprecipitation assay for anti-AChR antibodies(4) was negative on several occasions. The age at onset ranged between 2 and 68 years (median 24) and the duration of symptoms at sampling was between one month and 13 years (median 1.0 year). In 18 cases, plasma was obtained during therapeutic plasmapheresis which improved muscle strength. The remaining 6 samples were sera taken on first examination. Six of the patients had received corticosteroids for up to two months before sampling. Sera or plasmas were also obtained from healthy volunteers and from patients with anti-AChR antibody positive MG. IgG preparations were made using a Pierce ImmunoPureÒ (G) IgG purification kit.

MuSK and Agrin Expression Constructs

Constructs encoding full length MuSK(13) and the soluble fragment s-agrin (4/19)(20) have been described previously. MuSK deletion fragments comprising the entire extracellular domain (Ig1-4; aa 1-490, numbers according to ref (10)) or the first half encomprising two Ig-domains (Ig1-2: aa 1-230) were generated by insertion of artificial stop signals at these positions. N-terminal fragments of MuSK comprising the membrane-proximal extracellular domains, including Ig-domains 3 and 4 (Ig3-4: aa 198-430), or the transmembrane region and intracellular domain (MuSK D, aa 491-869) were generated. The corresponding c-DNA-fragments, including a newly introduced SphI-site, were linked to a vector containing an artificial signal sequence followed by six histidines and a 10aa epitope-tag (20). All constructs were transiently transfected into COS7 cells(12). For the production of soluble agrin and MuSK constructs, cells were switched to serum-free medium the second day after transfection. Conditioned media, containing MuSK or agrin fragments were removed 24 hours later and analyzed by Western blotting to confirm expression.

Immunostaining of MuSK-transfected COS7 Cells

COS7 cells were plated onto chamber slides the day after transfection. Two days later, cells were fixed with 2% paraformaldehyde and stained as described(13). Plasmas of myasthenia gravis patients and controls were analyzed in various dilutions (between 1:20 and 1:5000). Bound antibodies were visualized with secondary antibodies conjugated to Cy3 (anti-human IgG, Dianova). In all experiments, expression of transfected MuSK constructs was confirmed by staining parallel slides with rabbit-anti MuSK antibodies (13).

Immunoprecipitation Experiments

Detergent extracts were prepared from MuSK-transfected COS7 cells or from C2C12 myotubes that had been fused for five days. The immunoprecipitation was performed as described previously(12,13). AAAN and control plasmas incubated with the extracts at 1:20. Rabbit anti-MuSK serum was used at 1:100. MuSK in the immunoprecipitates was analysed by Western blotting using affinity-purified serum antibodies directed against the a MuSK cytoplasmic sequence(13).

ELISA Detection of Anti-MuSK Antibodies

Conditioned medium from MuSK-transfected COS-cells or from control cells mock-transfected with fish sperm DNA, was diluted 1:1 with 100 mM NaHCO3-buffer, pH 9.5 and applied overnight to ELISA plates. Plasmas were first tested at 1:5 in triplicates and subsequently at 1:10 in duplicates. Bound antibodies were detected by horse radish peroxidase-protein A (Amersham) followed by o-phenylenediamine and measuring A_{492}. For each sample, nonspecific immunoreactivity, determined by incubation of plates coated with conditioned medium from mock-transfected COS7 cells, was subtracted.

AChR Aggregation Assay

The mouse muscle cell line, C2C12, was used to determine functional effects of antibodies. Cells were plated onto chamber slides, fused and treated with or without agrin and/or plasmas or IgGs for five hours[13]. After fixation, AChRs were visualised with rhodaminea-bungarotoxin and the number of aggregates from more than 20 microscopic fields and at least two independent cultures were measured as described(20).

Results

We initially looked for IgG antibodies in five AAAN plasmas and three plasmas from healthy individuals using COS7 cells transfected with rat MuSK constructs (FIG. 1*a*). The experiments were performed blind. All five AAAN plasmas (eg FIG. 1*b*, AAAN), but none of the healthy control plasmas (eg HC), labelled MuSK aggregates on the cell surface at dilutions up to 1:1000. The pattern of immu-

noreactivity was indistinguishable from labelling observed with antibodies raised against recombinant MuSK in rabbits. (13) Each of the AAAN plasmas recognized the extracellular domains of MuSK, since no immunoreactivity was observed with COS7 cells expressing the transmembrane and cytoplasmic domains only (FIG. 1*b*, MuSK D). Not all cells expressed MuSK (compare FIG. 1*b*, AAAN/MuSK and Phase contrast, below), and these non-transfected cells and mock-transfected cells (not shown) did not bind the AAAN IgG antibodies.

Immunoprecipitation experiments confirmed that IgG antibodies in the AAAN plasmas recognized the native MuSK protein. Detergent extracts from MuSK-expressing COS7 cells and from mouse C2C12 myotubes, that express functional MuSK, were incubated with plasmas from two AAAN patients and a healthy control. Antibodies from both AAAN patients, but not from the control, immunoprecipitated bands of 110 kDa that were identified as MuSK by binding of a specific anti-MuSK antibody (FIG. 1*c*). With each extract, similar-sized bands were immunoprecipitated by a rabbit anti-MuSK serum from parallel extracts (FIG. 1*c*).

Sera and plasmas from AAAN, anti-AChR positive MG and healthy individuals were then tested in an ELISA. Fragments comprising only extracellular domains of MuSK were expressed in COS7 cells from which these soluble constructs are secreted, and the media were used as a source of the polypeptide antigen. IgG anti-MuSK antibodies, substantially greater than the mean+3SDs of the healthy control values (0.08 OD units) were found in 17/24 AAAN samples, whereas only borderline or negative values were found in the anti-AChR positive patients (FIG. 2*a*). Four of the seven negative, compared with only two of the 17 positive samples, were from patients who had received corticosteroid therapy before sampling.

Interestingly, in the 11 patients tested in both assays, the OD values for binding of antibodies to MuSK correlated ($p<0.02$) with IgG binding to the human TE671 cell line (which has features of human muscle) as measured previously(8). This suggests that MuSK is the target for AAAN IgG antibodies on the TE671 surface and that the negative values in seven samples are unlikely to be due to a lack of reactivity with rat MuSK Further results with four AAAN plasmas (eg FIG. 2*b*) indicated that the majority of antibodies are directed against the N-terminal sequences (construct Ig1-2 in FIG. 1*a*) and there was little reactivity with the membrane proximal half (construct Ig3-4 in FIG. 1*a*). We found no evidence of IgM antibodies to MuSK (data not shown), suggesting that the target for the putative non-IgG antibodies reported previously in some of the AAAN patients(15) will still need to be defined.

To investigate functional effects of the MuSK autoantibodies, we examined AChR clustering in myotubes derived from the mouse cell line, C2C12. In the absence of agrin (FIG. 3*a* upper panels), the control plasma produced very few clusters of AChRs (HC), whereas anti-MuSK positive plasma induced AChR, aggregates along the surface of the myotubes (AAAN). A similar antibody-induced induction of AChR-clustering by artificial dimerization of the kinase has previously been reported for rabbit antibodies induced against purified MuSK(13). Strikingly, when agrin was added with the plasmas (FIG. 3*a*, lower panels), the marked agrin-induced clustering which occurred in the presence of control plasma (HC) was not seen in the presence of AAAN plasma indicating that the anti-MuSK antibodies had inhibited the agrin-induced AChR clustering. Both the clustering (FIG. 3*b*) and the inhibitory activity (FIG. 3*c*) were found with each anti-MuSK positive plasmas or IgGs but not with anti-MuSK negative preparations. Since it is currently accepted that agrin does not bind directly to MUSK, but via a hypothetical agrin-binding component called MASC(1, 11), we speculate that the antibodies in AAAN patients bind to MuSK in such a manner as to prevent its interaction with MASC. This interaction is known to depend on the N-terminal half of the extracelluar domain of MuSK(16) which we find to be the main target for the IgG antibodies in anti AChR autoantibody negative patients (FIG. 2*b*).

To confirm the specificity of the test for myasthenia gravis, we tested a new group of controls (OND's) from patients with other neurological disorders. (FIG. 4). Only one serum was borderline positive. The relative incidence of MuSK antibodies in AAAN samples, was tested using a second cohort (Cohort 2) of *Myasthenia gravis* patients who were negative for acetylcholine receptor antibodies. All of these patients had generalised disease and 11/16 of them were positive for MuSK antibodies.

Antibodies to the fetal isoform of the acetylcholine receptor are found in a few mothers who have had babies born with complete paralysis and fixed joints (22,23). This severe condition is relatively common, but maternal antibodies to fetal acetylcholine receptor are found in only about 1% (Vincent, Dalton, unpublished findings). We asked whether MuSK antibodies might be present in some of these mothers. FIG. 5 shows, in comparison with the previously described results, that six mothers of affected babies out of a total of 200 tested (only 60 shown here) have these antibodies in their serum. This indicates that each of these six mothers has made an autoimmune response to MuSK and suggests that, after transfer of these antibodies across the placenta, they might be involved in causing the babies' condition. Testing for antibodies to MuSK in mothers of babies with muscle paralysis and/or fixed joints might indicate a fetal condition due to maternal antibodies.

To assess how the assay works out in practice, we have begun to compare results from patients with definite SNMG or a strong suspicion of SNMG with those in whom the diagnosis is questionable (?SNMG). FIG. 6 shows that among the first group, which includes cohort 1 and cohort 2, the assay is positive in 39/66 and among those with a questionable diagnosis the proportion is 6/25. The assay continues to be negative in healthy individuals.

The ELISA assay used as identified in the above example is difficult to standardise and we have tested an alternative assay, using immunoprecipitation of ^{125}I-MuSK. For this test, the purified extracellular domain of MuSK is iodinated using ^{125}I (carrier free from Amersham as for bungarotoxin in Ref (4, 6) or with chloramine T (standard conditions)). The iodinated MuSK is then separated from free ^{125}I by gel filtration. The ^{125}I-MuSK (approximately 50,000 cpm) is then added to 10 microlitres of the patient's serum over night. To immunoprecipitate the patients' antibodies and any ^{125}I-MuSK that is bound by them, excess of a sheep antibody to human IgG is added. The precipitate is centrifuged to form a pellet, washed and counted for radioactivity. The results (FIG. 7) show that healthy controls precipitated less than 1200 cpm, whereas 38/66 of the SNMG patients precipitated over 1200 cpm, the value rising to 7500 cpm which corresponds to approximately 1 nmole of MuSK precipitated per liter of serum. The assay was also positive in 5/25 patients with ?SNMG.

The results of the ELISA and immunoprecipitation assays were highly correlated (FIG. **8**). Most of the sera were positive with both assays or negative with both assays; there were three sera that gave negative results with the immunopreciptation and positive with ELISA, and two sera that were negative with the ELISA and positive with the immunoprecipitation.

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The invention claimed is:

1. A method for diagnosing neurotransmission or developmental disorders related to muscle specific tyrosine kinase (MuSK) in a mammal comprising the step of detecting in a bodily fluid of said mammal autoantibodies to an epitope of muscle specific tyrosine kinase (MuSK).

2. A method according to claim **1** wherein said method comprises the steps of:

a) contacting said bodily fluid with muscle specific tyrosine kinase (MuSK) or an antigenic determinant thereof; and

b) detecting any antibody-antigen complexes formed between said receptor tyrosine kinase or an antigenic fragment thereof and antibodies present in said bodily fluid, wherein the presence of said complexes is indicative of said mammal suffering from said neurotransmission or developmental disorders.

3. A method according to claim **2** wherein said antibody-antigen complex is detected using an anti-IgG antibody tagged or labeled with a reporter molecule.

4. A method according to claim **3** wherein said reporter molecule or label includes any of a heavy metal, a fluorescent or luminescent molecule, radioactive or enzymatic tag.

5. A method according to claim **4** wherein said enzymatic tag comprises horseradish peroxidase-protein A followed by reaction with o-phenylenediamine for subsequent measurement at A492.

6. A method according to claim **3** whereby the intensity of the signal from the anti-human IgG antibody is indicative of the relative amount of the anti-MuSK autoantibody in the bodily fluid when compared to a positive and negative control reading.

7. A method according to claim **1**, comprising contacting MuSK or an epitope or antigenic determinant thereof having a suitable label thereon, with said bodily fluid, immunoprecipitating any antibody/MuSK complex or antibody/MuSK epitope or antigenic determinant complex from said bodily fluid and monitoring for said label on any of said antibody/

MuSK complex or antibody/MuSK epitope or antigen determinant complex, wherein the presence of said label is indicative of said mammal is suffering from said neurotransmission or developmental disorder related to muscle specific tyrosine kinase (MuSK).

8. A method according to claim 7 wherein said label is a radioactive label.

9. A method according to claim 8 wherein said label is 125I.

10. A method according to claim 1 wherein said neurotransmission disorder is *Myasthenia gravis*.

11. A method according to claim 1, wherein said developmental disorder is muscle paralysis and/or fixed joints in newborn offspring due to maternal antibodies to MuSK.

12. A method for diagnosing neurotransmission or developmental disorders related to interference of the agrin/MuSK/AChR pathway within a mammal comprising the step of detecting in a bodily fluid of said mammal autoantibodies to an epitope of muscle specific tyrosine kinase (MuSK).

* * * * *

United States Court of Appeals for the Federal Circuit

ATHENA DIAGNOSTICS, INC., OXFORD UNIVERSITY INNOVATION LTD., MAX-PLANCK-GESELLSCHAFT ZUR FORDERUNG DER WISSENSCHAFTEN E.V.,
Plaintiffs-Appellants

v.

MAYO COLLABORATIVE SERVICES, LLC, DBA MAYO MEDICAL LABORATORIES, MAYO CLINIC,
Defendants-Appellees

2017-2508

Appeal from the United States District Court for the District of Massachusetts in No. 1:15-cv-40075-IT, Judge Indira Talwani.

Decided: February 6, 2019

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Before NEWMAN, LOURIE, and STOLL, *Circuit Judges.*

Opinion for the court filed by *Circuit Judge* LOURIE.

Dissenting opinion filed by *Circuit Judge* NEWMAN.

LOURIE, *Circuit Judge.*

Athena Diagnostics, Inc., Oxford University Innovation Ltd., and the Max-Planck-Gesellschaft zur Forderung der Wissenschaften E.V. (collectively, "Athena") appeal from the order of the United States District Court for the District of Massachusetts holding that claims 6–9 of U.S. Patent 7,267,820 (the "'820 patent") are invalid under 35 U.S.C. § 101 and dismissing Athena's complaint under Rule 12(b)(6). *Athena Diagnostics, Inc. v. Mayo Collaborative Servs., LLC*, 275 F. Supp. 3d 306 (D. Mass. 2017) ("*Decision*"). Because the district court correctly concluded that the claims at issue are directed to a natural law and lack an inventive concept, we affirm.

I. BACKGROUND

Athena Diagnostics is the exclusive licensee of the '820 patent, covering methods for diagnosing neurological disorders by detecting antibodies to a protein called muscle-specific tyrosine kinase ("MuSK"). '820 patent Abstract. Athena also markets a test called FMUSK that functions by evaluating those antibodies. After Mayo Collaborative Services, LLC ("Mayo") developed two competing tests that allegedly practice each step of one or more claims of the '820 patent, Athena accused Mayo of infringing its patent. Mayo moved to dismiss under Rule 12(b)(6), arguing that the asserted claims of the '820 patent were invalid under 35 U.S.C. § 101. The district court granted Mayo's motion, concluding that the claims were invalid under § 101 for claiming ineligible subject matter. This appeal solely concerns whether claims 6–9 are patent eligible under § 101.

A.

Myasthenia gravis ("MG") is a neurological disorder where patients experience muscle weakness and symptoms including drooping eyelids, double vision, and slurred speech. '820 patent col. 1 ll. 13–23. It was previously discovered that MG is an autoimmune disease caused by a

patient generating antibodies against her own acetylcholine receptors. *Id.* col. 1 ll. 24–26. Antibodies which recognize a person's own proteins as foreign antigens are known as autoantibodies. *Id.* col. 1 ll. 42–45.

About 80% of patients with MG produce acetylcholine receptor autoantibodies. *Id.* col. 1 ll. 34–36. The other 20% do not, but they do experience the same MG symptoms. *Id.* col. 1 ll. 36–38. The named inventors of the '820 patent discovered that many of the 20% of MG patients without acetylcholine receptor autoantibodies instead generate autoantibodies to a membrane protein called MuSK. *Id.* col. 1 ll. 54–61. Prior to their discovery, no disease had been associated with MuSK. *Id.* col. 2 ll. 35–37.

Having discovered the association between MuSK autoantibodies and MG, the inventors of the '820 patent disclosed and claimed methods of diagnosing neurological disorders such as MG by detecting autoantibodies that bind to a MuSK epitope.[1] *Id.* col. 2 ll. 61–65. Claim 1, not at issue in this appeal, is the only independent claim and reads as follows:

> 1. A method for diagnosing neurotransmission or developmental disorders related to [MuSK] in a mammal comprising the step of detecting in a bodily fluid of said mammal autoantibodies to an epitope of [MuSK].

Id. col. 12 ll. 31–35. Claim 7 is at issue and depends from claim 1. It recites:

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[1] An epitope, also known as an antigenic determinant, is a segment of a protein recognized by an antibody. *See* Bruce Alberts, *Molecular Biology of the Cell* 449–50 (6th ed. 2015). The specification of the '820 patent disclosed that autoantibodies in MG patients recognize a MuSK epitope located on the protein's extracellular amino-terminal domain. '820 patent col. 1 ll. 54–57.

> 7. A method according to claim 1, comprising
>
> contacting MuSK or an epitope or antigenic determinant thereof having a suitable label thereon, with said bodily fluid,
>
> immunoprecipitating any antibody/MuSK complex or antibody/MuSK epitope or antigenic determinant complex from said bodily fluid and
>
> monitoring for said label on any of said antibody/MuSK complex or antibody/MuSK epitope or antigen determinant complex,
>
> wherein the presence of said label is indicative of said mammal is suffering from said neurotransmission or developmental disorder related to [MuSK].

Id. col. 12 l. 62–col. 13 l. 5 (spacing added). Claim 8 depends from claim 7 and recites that the label is a radioactive label. *Id.* col. 13 ll. 6–7. Claim 9 depends from claim 8 and further recites that the radioactive label is 125I, a radioactive isotope of iodine. *Id.* col. 13 ll. 8–9. We focus on claim 9, the most specific one at issue, which requires: (1) contacting MuSK or an epitope thereof having a 125I label, with bodily fluid; (2) immunoprecipitating any antibody/MuSK complex; and (3) monitoring for the label on the complex, wherein the presence of the label indicates the presence of a MuSK-related disorder.

The specification of the '820 patent further explains what the steps of iodination and immunoprecipitation entail. First, MuSK is iodinated using radioactive 125I. *Id.* col. 10 ll. 50–52. Then iodinated MuSK is separated from any free 125I by gel filtration. *Id.* col. 10 ll. 55–56. Next, the 125I-labeled MuSK is added to a small volume of the patient's bodily fluid and left overnight. *Id.* col. 10 ll. 56–58. If MuSK autoantibodies are present in the patient's bodily fluid, they will bind to the 125I-labeled MuSK. Any 125I-labeled MuSK in the sample is then immunoprecipitated by adding a secondary antibody that binds to any

MuSK autoantibodies present. *Id.* col. 10 ll. 58–60. The resulting precipitate is finally centrifuged, washed, and counted for radioactivity, which may be indicative of MG. *Id.* col. 10 ll. 60–61.

It is undisputed that iodination and immunoprecipitation were known techniques at the time of the invention. The '820 patent specification states that "[t]he actual steps of detecting autoantibodies in a sample of bodily fluids may be performed in accordance with immunological assay techniques known per se in the art," such as radioimmunoassays. *Id.* col. 3 ll. 33–37. With respect to the relevant individual steps in the radioimmunoassay, the specification also discloses that "[i]odination and immunoprecipitation are standard techniques in the art." *Id.* col. 4 ll. 10–11.

Claim 6 is additionally at issue in this appeal and depends from claim 3. While claim 6 also involves detecting MuSK autoantibodies by contacting a patient's bodily fluid with MuSK or an epitope thereof, the labelling occurs somewhat differently than in claims 7–9. Instead of labeling MuSK with a radioisotope, claim 3 recites that the secondary antibody is "tagged or labeled with a reporter molecule." *Id.* col. 12 ll. 47–49. Claim 6 additionally requires that "the intensity of the signal from the [secondary] antibody is indicative of the relative amount of the anti-MuSK autoantibody in the bodily fluid when compared to a positive and negative control reading." *Id.* col. 12 ll. 57–61. This claimed technique exemplifies the ELISA method,[2] which, like radioimmunoassays, the '820 patent specification lists as an example of "immunological assay techniques known per se in the art." *Id.* col. 3 ll. 33–36.

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[2] ELISA stands for enzyme-linked immunosorbent assay. The technical details of this assay are not relevant to this appeal.

B.

The district court concentrated its analysis on claims 7–9. Athena did not present any arguments specific to claim 6. Applying the test for subject matter eligibility established by the Supreme Court in *Mayo Collaborative Services v. Prometheus Laboratories, Inc.*, 566 U.S. 66 (2012) and *Alice Corp. v. CLS Bank International*, 573 U.S. 208 (2014), the court first concluded that the claims were directed to a law of nature, *Decision*, 275 F. Supp. 3d at 312. According to the court, the claims focused on the interaction of 125I-labeled MuSK with MuSK autoantibodies in bodily fluid, an interaction which occurs naturally. *Id.* at 310. The district court also determined that the claims lacked an inventive concept, as the recited steps involved only standard techniques in the art. *Id.* at 312–13.

The district court thus dismissed Athena's complaint for failure to state a claim. Athena appealed. We have jurisdiction under 28 U.S.C. § 1295(a)(1).

II. DISCUSSION

We review the district court's dismissal for failure to state a claim under regional circuit law. *BASCOM Glob. Internet Servs., Inc. v. AT&T Mobility LLC*, 827 F.3d 1341, 1347 (Fed. Cir. 2016). The First Circuit reviews such dismissals *de novo*, accepts all well-pleaded facts alleged in the complaint to be true, and draws all reasonable inferences in favor of the non-movant. *In re Loestrin 24 Fe Antitrust Litig.*, 814 F.3d 538, 549 (1st Cir. 2016). Patent eligibility under § 101 is a question of law based on underlying facts, *see Aatrix Software, Inc. v. Green Shades Software, Inc.*, 882 F.3d 1121, 1125 (Fed. Cir. 2018); *Berkheimer v. HP Inc.*, 881 F.3d 1360, 1364–65 (Fed. Cir. 2018), that may be resolved on a Rule 12(b)(6) motion when the undisputed facts require a holding of ineligibility, *SAP Am., Inc. v. Investpic, LLC*, 898 F.3d 1161, 1166 (Fed. Cir. 2018).

Section 101 provides that "[w]hoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title." 35 U.S.C. § 101. Given the expansive terms of § 101, "Congress plainly contemplated that the patent laws would be given wide scope"; some of the legislative history likewise indicated that "Congress intended statutory subject matter to 'include anything under the sun that is made by man.'" *Diamond v. Chakrabarty*, 447 U.S. 303, 308–09 (1980).

Under the law as set forth by the Supreme Court, § 101, while broad, "contains an important implicit exception. '[L]aws of nature, natural phenomena, and abstract ideas' are not patentable." *Mayo*, 566 U.S. at 70 (alteration in original) (quoting *Diamond v. Diehr*, 450 U.S. 175, 185 (1981)). These exceptions exist because monopolizing the basic tools of scientific work "might tend to impede innovation more than it would tend to promote it." *Id.* at 71. However, the Supreme Court has advised that these exceptions must be applied cautiously, as "too broad an interpretation of this exclusionary principle could eviscerate patent law." *Id.*

Laws of nature are not patentable, but applications of such laws may be patentable. A claim to otherwise statutory subject matter does not become ineligible by its use of a law of nature. *See Diehr*, 450 U.S. at 187; *Parker v. Flook*, 437 U.S. 584, 590 (1978). But, on the other hand, adding "conventional steps, specified at a high level of generality," to a law of nature does not make a claim to the law of nature patentable. *Mayo*, 566 U.S. at 82.

To distinguish claims to patent-eligible applications of laws of nature from claims that impermissibly tie up such laws, we apply the two-part test set forth by the Supreme Court. First, we examine whether the claims are "directed to" a law of nature. *Alice*, 573 U.S. at 217. If they are, then

we proceed to the second inquiry, where we ask whether the limitations of the claim apart from the law of nature, considered individually and as an ordered combination, "'transform the nature of the claim' into a patent-eligible application." *Id.* (quoting *Mayo*, 566 U.S. at 78). To so transform the claim, the additional limitations must "ensure that the patent in practice amounts to significantly more than a patent upon the natural law itself." *Mayo*, 566 U.S. at 73.

We first address claims 7–9 and then turn to claim 6.

A.

Athena argues that claims 7–9 are not directed to a natural law at step one because they recite innovative, specific, and concrete steps that do not preempt a natural law. Rather, Athena contends that the claims are directed to a new laboratory technique that makes use of man-made molecules.

Mayo responds that the claims are directed to a natural law: the correlation between naturally-occurring MuSK autoantibodies and MuSK-related neurological diseases like MG. According to Mayo, the remaining steps apart from the natural law are concededly standard immunoassay techniques that still leave the claim directed to a natural law. Indeed, Mayo argues that the specificity and concreteness of the claimed steps are irrelevant to whether a claim is directed to a natural law. And, as in *Mayo*, Mayo contends that it makes no difference to eligibility that the claimed diagnostic method uses man-made materials.

We ultimately agree with Mayo that, under *Mayo*, the claims are directed to a natural law. As an initial matter, we must identify what the relevant natural law is. Here, it is the correlation between the presence of naturally-occurring MuSK autoantibodies in bodily fluid and MuSK-

related neurological diseases like MG.[3] This correlation exists in nature apart from any human action. There can thus be no dispute that it is an ineligible natural law.

However, as Athena correctly observes, not every claim that involves a natural law is directed to a natural law. "[A]ll inventions at some level embody, use, reflect, rest upon, or apply laws of nature, natural phenomena, or abstract ideas." *Mayo*, 566 U.S. at 71. The Supreme Court's two-step test thus "plainly contemplates that the first step of the inquiry is a meaningful one, i.e., that a substantial class of claims are *not* directed to a patent-ineligible concept." *Enfish, LLC v. Microsoft Corp.*, 822 F.3d 1327, 1335 (Fed. Cir. 2016).

The step one "directed to" inquiry focuses on the claim as a whole. *E.g.*, *Elec. Power Grp., LLC v. Alstom S.A.*, 830 F.3d 1350, 1353 (Fed. Cir. 2016). To determine whether a claim is directed to an ineligible concept, we have frequently considered whether the claimed advance improves upon a technological process or merely an ineligible concept, based on both the written description and the claims. *See Cleveland Clinic Found. v. True Health Diagnostics LLC*, 859 F.3d 1352, 1361 (Fed. Cir. 2017); *Rapid Litig. Mgmt. Ltd. v. CellzDirect, Inc.*, 827 F.3d 1042, 1047–49 (Fed. Cir. 2016); *Ariosa Diagnostics, Inc. v. Sequenom, Inc.*, 788 F.3d 1371, 1376 (Fed. Cir. 2015); *see also McRO, Inc. v.*

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[3] We note that the district court held that the "focus of the claims" was the binding of MuSK to MuSK antibodies in bodily fluid. *Decision*, 275 F. Supp. 3d at 310. Our cases have not described a claim to the binding of two molecules during a sequence of chemical manipulations (here, after MuSK labeling and before immunoprecipitation) as a claim to a natural law, even if such binding occurs according to natural laws. We need not resolve that issue here, as we agree with Mayo's identification of the natural law.

Bandai Namco Games Am. Inc., 837 F.3d 1299, 1314–15 (Fed. Cir. 2016); *Elec. Power Grp.*, 830 F.3d at 1354.

For example, in *CellzDirect* we considered claims that covered a method for producing a preparation of a type of liver cell (called hepatocytes) that involved multiple freeze-thaw cycles. 827 F.3d at 1046, 1048. Although the inventors discovered the cells' ability to survive multiple freeze-thaw cycles, a discovery that the district court understood to be a natural law, we concluded that the claims were not directed to that natural law. *Id.* at 1048–50. This was because the claims as a whole recited "a new and improved way of preserving hepatocyte cells for later use," "not simply an observation or detection of the ability of hepatocytes to survive multiple freeze-thaw cycles." *Id.* at 1048. The claimed advance harnessed a natural law to produce a technological improvement that was patent eligible. *See id.* at 1048–49; *see also, e.g.*, *Enfish*, 822 F.3d at 1335–39 (holding improvement in computer-related technology not directed to abstract idea).

In contrast, in *Cleveland Clinic* we reiterated that claims that merely recite observing naturally occurring biological correlations "with no meaningful non-routine steps in between" are directed to a natural law. 859 F.3d at 1361; *see Ariosa*, 788 F.3d at 1376. There, the specification indicated that the claimed inventors discovered a natural correlation between a molecule called MPO and cardiovascular disease. *Cleveland Clinic*, 859 F.3d at 1360–61. The claims at issue recited detecting MPO or other MPO-related products in a patient sample and then predicting a patient's risk of having or developing cardiovascular disease. *Id.* at 1361. As the claims only covered the correlation between MPO and cardiovascular disease, an ineligible discovery, together with "well-known techniques to execute the claimed method," we held that the claims were directed to a natural law. *Id.*

The claims at issue here involve both the discovery of a natural law and certain concrete steps to observe its operation. Claim 9, the most specific claim at issue, recites the following method to detect MuSK autoantibodies: (1) mixing MuSK or an epitope thereof having a 125I label with bodily fluid; (2) immunoprecipitating any resulting antibody/MuSK complex; and (3) monitoring for the label on the complex. '820 patent col. 12 l. 62–col. 13 l. 9. The claim then concludes in the wherein clause with a statement of the natural law, *i.e.*, the discovery that MuSK autoantibodies naturally present in a patient sample, detected with the 125I label bound to the MuSK/antibody complex, indicate that the patient is suffering from a MuSK-related neurological disorder. *Id.* col. 13 ll. 2–5.

As in *Cleveland Clinic* and *Ariosa*, we conclude that claims 7–9 are directed to a natural law because the claimed advance was only in the discovery of a natural law, and that the additional recited steps only apply conventional techniques to detect that natural law. The specification of the '820 patent highlights the discovery of the natural law, explaining that "[t]he present inventors surprisingly found that many of the 20% of MG patients [who] do not exhibit any autoantibodies to [the acetylcholine receptor], instead have . . . antibodies directed against the extracellular [amino]-terminal domains of MuSK." *Id.* col. 1 ll. 54–57. Further, the specification describes the claimed concrete steps for observing the natural law as conventional. It teaches that "[t]he actual steps of detecting autoantibodies in a sample of bodily fluids may be performed in accordance with immunological assay techniques known per se in the art," including radioimmunoassays and ELISA. *Id.* col. 3 ll. 33–37. Likewise, the specification identifies "[i]odination and immunoprecipitation" as "standard techniques in the art." *Id.* col. 4 ll. 10–12. The '820 patent thus describes the claimed invention principally as a discovery of a natural law, not as an improvement in the underlying immunoassay technology.

Consistent with the specification, the claims are directed to that law.

Athena argues that the claims at issue, like the claims in *CellzDirect*, are directed to an innovative laboratory technique, not a law of nature. However, Athena does not point to any innovation other than its discovery of the natural law. *CellzDirect* did not suggest that appending standard techniques to detect a natural law rendered claims not directed to a natural law; rather, we expressly distinguished the eligible claims in that case from ineligible claims that "amounted to nothing more than observing or identifying the ineligible concept itself." 827 F.3d at 1048. In that case, we concluded that the "end result" of the claims at issue was "not simply an observation or detection" of a natural law. *Id.* We cannot so conclude here, since the claims before us only involve detecting a natural law "with no meaningful non-routine steps." *Cleveland Clinic*, 859 F.3d at 1361.

Athena also points to the specificity of the claimed concrete steps, contending that they preempt no natural law and therefore the claims cannot be directed to a natural law. Although we agree that claim 9 leaves open to the public other ways of interrogating the correlation between MuSK autoantibodies and MuSK-related disorders without practicing the claim's concrete steps, that does not disturb our conclusion at step one. Preemption is sufficient to render a claim ineligible under § 101, but it is not necessary. *Flook*, 409 U.S. at 71–72 (holding claim involving mathematical formula invalid under § 101 that did not preempt a mathematical formula); *Ariosa*, 788 F.3d at 1379; *In re BRCA1- & BRCA2-Based Hereditary Cancer Test Patent Litig.*, 774 F.3d 755, 764 n.4 (Fed. Cir. 2014). The claims here are directed to a natural law because they recite only the natural law together with standard techniques for observing it. That the routine steps are set forth with some specificity is not enough to change that conclusion.

Finally, Athena argues that the claims at issue differ from prior diagnostic claims we have held ineligible under § 101 because they require labeling MuSK with a man-made substance. We disagree. As Mayo argues, the use of a man-made molecule is not decisive if it amounts to only a routine step in a conventional method for observing a natural law. For example, *Mayo* involved claims requiring administering a man-made molecule (a drug "providing" 6-thioguanine) to a patient. 566 U.S. at 74–75. Some of the claims in *Ariosa* likewise required amplification through the polymerase chain reaction, which makes use of man-made reagents, *see* U.S. Patent 6,258,540 col. 5 ll. 6–26, or using a specific probe that binds to DNA, 788 F.3d at 1374. And the claims in *BRCA1* also involved hybridizing a synthetic DNA probe to a DNA strand. *BRCA1*, 774 F.3d at 763–64. Nonetheless, in each of these cases either the Supreme Court or this court held the claims directed to a natural law and invalid under § 101. *Mayo*, 566 U.S. at 92; *Ariosa*, 788 F.3d at 1380; *BRCA1*, 774 F.3d at 765. We thus reaffirm that use of a man-made molecule in a method claim employing standard techniques to detect or observe a natural law may still leave the claim directed to a natural law.

We consider it important at this point to note the difference between the claims before us here, which recite a natural law and conventional means for detecting it, and applications of natural laws, which are patent-eligible. *See Vanda Pharm. Inc. v. West-Ward Pharm. Int'l Ltd.*, 887 F.3d 1117, 1133–36 (Fed. Cir. 2018) (holding that method of treatment by administering drug at certain dosage ranges based on a patient's genotype was not directed to a natural law). Claiming a natural cause of an ailment and well-known means of observing it is not eligible for patent because such a claim in effect only encompasses the natural law itself. But claiming a new treatment for an ailment, albeit using a natural law, is not claiming the natural law.

As we conclude that claims 7–9 are directed to a natural law, we turn to the second step of the *Mayo/Alice* test.[4]

B.

At step two, "we consider the elements of each claim both individually and 'as an ordered combination' to determine whether the additional elements 'transform the nature of the claim' into a patent-eligible application." *Alice*,

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[4] The dissent states much that one can agree with from the standpoint of policy, and history, including that "the public interest is poorly served by adding disincentive to the development of new diagnostic methods." Dissent at 12. We would add further that, in our view, providing patent protection to novel and non-obvious diagnostic methods would promote the progress of science and useful arts. But, whether or not we as individual judges might agree or not that these claims only recite a natural law, *cf. Berkheimer v. HP Inc.*, 890 F.3d 1369, 1374 (Fed. Cir. 2018) (Lourie, J., concurring in the denial of rehearing en banc) (discussing traditional laws of nature such as "Ohm's Law, Boyle's Law, [and] the equivalence of matter and energy"), the Supreme Court has effectively told us in *Mayo* that correlations between the presence of a biological material and a disease are laws of nature, *see* 566 U.S. at 77, and "[p]urely 'conventional or obvious' '[pre]-solution activity' is normally not sufficient to transform an unpatentable law of nature into a patent-eligible application of such a law," *id.* at 79 (second alteration in original) (quoting *Flook*, 437 U.S. at 590). We have since confirmed that applying somewhat specific yet conventional techniques (such as the polymerase chain reaction) to detect a newly discovered natural law does not confer eligibility under § 101. *Ariosa*, 788 F.3d at 1377; *see also Cleveland Clinic*, 859 F.3d at 1356, 1362 (addressing other conventional techniques such as flow cytometry). Our precedent leaves no room for a different outcome here.

573 U.S. at 217 (quoting *Mayo*, 566 U.S. at 78, 79). "Purely 'conventional or obvious' '[pre]-solution activity' is normally not sufficient to transform an unpatentable law of nature into a patent-eligible application of such a law." *Mayo*, 566 U.S. at 79 (second alteration in original) (quoting *Flook*, 437 U.S. at 590). The transformative "inventive concept" supplied by the claim elements not drawn to ineligible subject matter must be "sufficient to ensure that the patent in practice amounts to significantly more than a patent upon the [ineligible concept] itself." *Alice*, 573 U.S. at 217–18 (quoting *Mayo*, 566 U.S. at 73).

1.

Athena argues that the claims provide an inventive concept: an innovative sequence of steps involving man-made molecules. Prior to its discovery, Athena contends that there was no disclosed method to detect MuSK autoantibodies. In addition, Athena argues that the existence of factual disputes precluded dismissal under Rule 12(b)(6).

Mayo responds that the claims lack an inventive concept because the specification describes the steps for detecting MuSK autoantibodies as standard techniques in the art. Furthermore, Mayo argues that no factual issues precluded the district court's dismissal under Rule 12(b)(6).

We agree with Mayo that the steps of the claims not drawn to ineligible subject matter, whether viewed individually or as an ordered combination, only require standard techniques to be applied in a standard way. As previously discussed, the specification of the '820 patent plainly states that "[t]he actual steps of detecting autoantibodies in a sample of bodily fluids may be performed in accordance with immunological assay techniques known per se in the art," such as radioimmunoassays. '820 patent col. 3 ll. 33–37. Iodination and immunoprecipitation are likewise described as standard techniques. *Id.* col. 4 ll. 9–12. Because the specification defines the individual immunoprecipitation and iodination steps and the overall

radioimmunoassay as conventional techniques, the claims fail to provide an inventive concept. *Cleveland Clinic*, 859 F.3d at 1362; *Ariosa*, 788 F.3d at 1378.

Our decisions in *CellzDirect* and *BASCOM* are consistent with the principle that applying standard techniques in a standard way to observe a natural law does not provide an inventive concept. In *CellzDirect*, we considered a combination of claimed steps involving two freeze/thaw cycles. 827 F.3d at 1051. We held that this combination of steps was not conventional because the prior art methods only disclosed using one freeze/thaw cycle and, in fact, taught away from using multiple freeze/thaw cycles. *Id.* Similarly, in *BASCOM* we held that the ordered combination of claim limitations was not routine and conventional because they placed a filtering tool at a specific location that improved on prior art technology. 827 F.3d at 1350. The inventive concept was "found in the non-conventional and non-generic arrangement of known, conventional pieces." *Id.* In contrast, claims 7–9 of the '820 patent employ a conventional technique for detecting autoantibodies, a radioimmunoassay, which the specification acknowledges was "known per se in the art." '820 patent col. 3 ll. 33–37. The individual constituent steps of that technique, iodination and immunoprecipitation, are similarly described as standard. *Id.* col. 4 ll. 9–12. Thus, unlike the claimed limitations at issue in *CellzDirect* and *BASCOM*, the recited steps here were conventional both as an ordered combination and individually.

Athena also argues that the claimed steps were unconventional because they had not been applied to detect MuSK autoantibodies prior to Athena's discovery of the correlation between MuSK autoantibodies and MG. Even accepting that fact, we cannot hold that performing standard techniques in a standard way to observe a newly discovered natural law provides an inventive concept. This is because "[t]he inventive concept necessary at step two . . . cannot be furnished by the unpatentable law of nature

. . . itself." *Genetic Techs. Ltd. v. Merial L.L.C.*, 818 F.3d 1369, 1376 (Fed. Cir. 2016); *see Mayo*, 566 U.S. at 73 (considering whether the "claimed processes (apart from the natural laws themselves)" were routine and conventional). Rather, to supply an inventive concept the sequence of claimed steps must do more than adapt a conventional assay to a newly discovered natural law; it must represent an inventive application beyond the discovery of the natural law itself. Because claims 7–9 fail to recite such an application, they do not provide an inventive concept.

Similar to its step one argument, Athena further argues that the claims recite an inventive concept because they use a man-made molecule, *i.e.*, labeled MuSK. Athena analogizes its methods involving labeled MuSK to the composition claims involving cDNA held eligible in *Association for Molecular Pathology v. Myriad Genetics, Inc.*, 569 U.S. 576, 594–95 (2013). However, the method claims at issue here are unlike the claims held eligible in *Myriad*, which recited a new composition of matter that was not a natural product. *Id.* For the same reasons that we have concluded that attaching a label to MuSK did not make the claims directed to an eligible concept at step one, we conclude that appending labeling techniques to a natural law does not provide an inventive concept where, as here, the specification describes 125I labeling as a standard practice in a well-known assay.

2.

Athena also argues that the district court needed to conduct fact-finding before resolving the § 101 issue. But, unlike in *Aatrix*, 882 F.3d at 1128, Athena directs us to no factual allegations in its complaint—amended three times—that the radioimmunoassay technique recited in claims 7–9 is anything other than standard and "known per se in the art." '820 patent, col. 3 ll. 33–37. Instead, Athena relies on an expert declaration submitted with its opposition to Mayo's motion to dismiss, asserting that iodination

and immunoprecipitation were not routine as applied to the claimed invention. In dismissing Athena's complaint under Rule 12(b)(6), the district court did not consider the declaration. Athena argues that was error. We disagree.

In the First Circuit, under Rule 12(b)(6) a district court may generally "consider only facts and documents that are part of or incorporated into the complaint; if matters outside the pleadings are considered, the motion must be decided under the more stringent standards applicable to a Rule 56 motion for summary judgment." *Trans-Spec Truck Serv., Inc. v. Caterpillar Inc.*, 524 F.3d 315, 321 (1st Cir. 2008). Certain documents, like the '820 patent here, are also considered to "merge[] into the pleadings" where the "complaint's factual allegations are expressly linked to" and dependent upon a document, the authenticity of which is undisputed. *Id.* (quoting *Beddall v. State St. Bank & Trust Co.*, 137 F.3d 12, 16–17 (1st Cir. 1998)).

District courts in the First Circuit have discretion whether to convert a motion to dismiss into a motion for summary judgment. *Id.* (citing Fed. R. Civ. P. 12(d)). "[I]f the district court chooses . . . to ignore supplementary materials submitted with the motion papers and determine the motion under the Rule 12(b)(6) standard, no conversion occurs and the supplementary materials do not become part of the record for purposes of the Rule 12(b)(6) motion." *Id.*

We conclude that the district court did not abuse its discretion in declining to consider Athena's expert declaration and convert the motion into one for summary judgment. The declaration does not "merge into the pleadings," as the complaint does not reference it or otherwise depend on it. Nor is the declaration an official public record, another type of document a court may consider with the pleadings. *See Watterson v. Page*, 987 F.2d 1, 3–4 (1st Cir. 1993).

Athena does not expressly argue that the district court abused its discretion, but does contend, primarily citing non-binding authority, that the plaintiff may freely allege facts without support in responding to a motion to dismiss as long as those facts are consistent with the complaint, *see Early v. Bankers Life & Casualty Co.*, 959 F.2d 75, 79 (7th Cir. 1992), and that its expert declaration alleged such consistent facts that create a dispute of material fact.

Even assuming this general principle applies in the First Circuit—an assumption that Athena meagerly supports—the district court did not need to consider the allegations in the expert declaration because they were not consistent with the complaint read in light of the '820 patent. These technical allegations include: (1) that detecting MuSK autoantibodies required the "creative step" of breaking up MuSK into smaller fragments, J.A. 623, 625; (2) that identifying a specific site on MuSK to label would not have been routine because many factors contribute to whether a binding site for a label is adequate, J.A. 626–28; and (3) that immunoprecipitation is generally uncertain and not routine, J.A. 630. None of these details are recited in the claims of the '820 patent: no claim requires breaking MuSK into fragments as opposed to using the entire MuSK protein; no claim is limited to a particular MuSK binding site; and no claim recites any detail with respect to immunoprecipitation. Those omissions are consistent with the specification's description of iodination, immunoprecipitation, and the overall radioimmunoassay as standard techniques. Because Athena's expert declaration made allegations inconsistent with the '820 patent, the district court was not obliged to accept them as true. For these reasons, the district court did not err in dismissing Athena's complaint under Rule 12(b)(6).

C.

Claim 6 recites a method for detecting MuSK autoantibodies different from claims 7–9. While claims 7–9 recite

a radioimmunoassay, claim 6 recites an ELISA method. Like radioimmunoassays, the specification describes ELISA as an "immunological assay technique[] known per se in the art." '820 patent col. 3 ll. 32–36. The main technical difference pertinent to this appeal between an ELISA and a radioimmunoassay is that in an ELISA, the secondary antibody rather than the antigen is labeled.

Athena argues that since the district court did not specifically analyze claim 6, which involves a different technology, and implicitly treated claims 7–9 as representative, we should remand at least with respect to claim 6. Mayo responds that the district court properly grouped claim 6 with claims 7–9 because Athena grouped them together, and that Athena waived any separate arguments regarding claim 6 by not specifically addressing that claim in its briefing.

During the district court proceedings, Athena represented that it would not assert claims 1–5 and 10–12, and Mayo then moved to dismiss Athena's complaint, specifically addressing claims 6–9. In its response, Athena did not make any particularized arguments regarding claim 6, and, in an earlier response, indicated that the same arguments pertaining to claims 7–9 were also applicable to claim 6. *See* J.A. 180 ("While the claim does not require radioactive MuSK or complexes, many other arguments relating to claims 7-9 apply to claim 6."). The district court did not address claim 6 in its order beyond listing it among the other claims. *Decision*, 275 F. Supp. 3d at 309–10.

Given this history, we agree with Mayo that Athena waived its arguments specific to claim 6 by not making them before the district court. We apply regional circuit law to the issue of waiver, as it is not unique to patent law. *Riverwood Int'l Corp. v. R.A. Jones & Co.*, 324 F.3d 1346, 1352 (Fed. Cir. 2003) (citing *Midwest Indus., Inc. v. Karavan Trailers, Inc.*, 175 F.3d 1356, 1359 (Fed. Cir. 1999) (en banc in relevant part)). In the First Circuit, an argument

may be deemed waived that was not presented to the district court. *Butler v. Deutsche Bank Tr. Co. Ams.*, 748 F.3d 28, 36 (1st Cir. 2014). Although Athena recognized that claim 6 was at issue, it concededly did not present any specific arguments concerning the eligibility of claim 6. Appellant's Br. 15. It was not incumbent on the district court to address arguments that Athena did not make. We thus find no error in the district court considering claims 7–9 as representative of claim 6. Even if we had reached the issue, we would hold claim 6 ineligible. The specification describes ELISA as an "immunological assay technique[] known per se in the art." '820 patent col. 3 ll. 32–36. Claim 6 merely recites the application of this standard technique to observe a natural law. This does not provide an inventive concept under step two.

CONCLUSION

We have considered Athena's remaining arguments but find them unpersuasive. Because claims 6–9 of the '820 patent recite only a natural law together with conventional steps to detect that law, they are ineligible under § 101. For the foregoing reasons, we affirm the judgment of the district court.

AFFIRMED

Athena v. Mayo: A Splintered Federal Circuit Invites Supreme Court or Congress to Step Up On 101 Chaos

By **IPWatchdog**
July 8, 2019

Pr

"Unless one opposes the notion of patent protection entirely, it cannot be reasonably disputed that claims to diagnostic kits and techniques, like pharmaceuticals, which require enormous initial investments in terms of both time and money, are the reason we suffer the promise of a monopoly." – Federal Circuit Judge Kimberly Moore

On July 3, the Court of Appeals for the Federal Circuit denied *en banc* rehearing in *Athena Diagnostics v. Mayo Collaborative Services*. The 86-page order from the Federal Circuit includes eight separate opinions—four concurring with the *en banc* denial and another four dissenting from the decision. The separate opinions reflect a Federal Circuit that isn't divided so much on the issue of the importance of Athena's now invalidated patent claims but, rather, the application of the U.S. Supreme Court's Section 101 jurisprudence under *Mayo Collaborative Services v. Prometheus Laboratories* (2012). Throughout the opinions, it seemed clear that the Federal Circuit was eager to have the Supreme Court take this case up on appeal in order to clarify *Mayo*'s judicial exception to laws of nature and its impact on patent claims covering medical diagnostics.

Judge Dyk's Concurrence, Joined by Judge Hughes and, In Part, by Judge Chen

The concurrence by Judge Timothy Dyk, which was fully joined by Judge Todd Hughes, spoke approvingly of the *Mayo/Alice* subject matter eligibility framework, which has been "valuable and effective" at invalidating overly broad claims in a way that cannot be accomplished by Section 102 novelty, Section 103 obviousness or Section 112 enablement or written description. Dyk shared his

colleagues' concerns that the *Mayo* test should leave room for diagnostic patents that are sufficiently specific, but found that "it is the Supreme Court, not this court, that should reconsider the breadth of *Mayo*." That decision didn't make all diagnostic claims patent-ineligible, but it "left no room for us to find typical diagnostic claims patent eligible, absent some inventive concept at *Mayo* step two," Dyk wrote.

Judge Raymond Chen joined Judge Dyk's concurrence at Parts VI, V and IV. In this portion, Dyk pointed out that there is tension between *Mayo* and the Supreme Court's holding in *Association for Molecular Pathology v. Myriad Genetics* (2013), which supports the patentability of discoveries under Section 101 as long as the claims aren't drafted overbroadly. Judge William Bryson's concurrence in *Myriad* noted claims unchallenged in the lower court's decision, recognizing that the potential eligibility of those claims led to the notion that "an inventive concept can sometimes come from the discovery of an unknown natural phenomenon and its application for a diagnostic purpose." While Judge Dyk felt that the Supreme Court should refine *Mayo*, such refinement should be limited to preventing the claiming of natural laws by tying those laws to a specific and useful application at *Mayo* step one, which could serve as the determination of the "inventive concept" in *Mayo* step two. Under that approach, Athena's claims could be determined to be patent eligible, as they don't claim a natural law itself but rather specific methods of diagnosing a neurological disorder by detecting certain antibodies. Unlike *Mayo*, this case involved the discovery of that relationship and "not mere determination of the precise correlations of a known natural law using prior art processes."

Judge Chen's Concurrence

Judge Chen wrote his own concurrence in the *en banc* denial in which he spent time discussing the different Supreme Court holdings in *Parker v. Flook* (1978) and *Diamond v. Diehr* (1981). In *Flook*, the Court required an inventive concept in claims beyond the recitation of an algorithm or law of nature after analyzing the claims on an element-by-element basis. By contrast, the Court in *Diehr* required the analysis of the challenged patent claim as a whole. *Diehr* served as the guiding precedent on the Section 101 inquiry for three decades until *Mayo*, which employed reasoning that tracked both *Flook* and Justice John Paul Stevens' dissent in *Diehr*. Chen argued that *Alice Corp. v. CLS Bank International* (2014) marked a further reversion to *Flook*'s analytical framework of considering elements of patent claims individually.

Judge Chen did note that *Mayo*'s analytical framework is harder to apply consistently than *Diehr* "and more aggressive in its reach." Further, *Mayo* didn't expressly overturn *Diehr*'s limitation on *Flook* that emphasized the need to consider the invention as a whole. Chen's discussion of this point clearly invites Supreme Court review of this case:

> *Through it all, there is a serious question today in patent law as to what extent Diehrremains good law in light of Mayo. We are not in a position to resolve that question, but the Supreme Court can. Resolution of the present confusion is important because if Mayo in fact overruled*

> *the principles in Diehr… then that would be a significant incursion on the settled expectations that had existed for 30 years since Diehr.*

Athena provides ample proof of this present confusion. Judge Chen noted that the dissents and *amici* raise several valid concerns but, under *Mayo*, Athena's patent claims involving the association of an antibody and a medical disorder is deemed to be a law of nature and not an application of that law. Additional claim elements such as label-adding steps, immunoprecipitating steps or the use of a particular label are all conventional and add nothing to the underlying technology. While new diagnostic methods "intuitively seem to be the kind of subject matter the patent system is designed for," the claims don't stand under *Mayo*'s scrutiny, but Chen indicated that they could be patentable if read "as a whole" under *Diehr*. Chen also noted that Athena's claims arguably cover a new use of a known composition of matter, which the Patent Act contemplates as a patentable discovery; the Supreme Court hasn't addressed the meaning of "discovers" separately from "invents" in Section 101.

Judge Moore's Dissent, Joined by Judges O'Malley, Wallach and Stoll

Judge Kimberly Moore'd dissent, the lengthiest opinion in the *en banc* denial, started by noting that none of the judges of the Federal Circuit agree that the claims should be ineligible. The only disagreement is whether *Mayo* requires that outcome. Since *Mayo*, Moore noted that the Federal Circuit has invalidated every diagnostic claim that has come before the court in eight separate cases. "We have turned *Mayo* into a per se rule that diagnostic kits and techniques are ineligible," Moore wrote. When reading *Mayo* in light of *Myriad*, Moore felt it was clear that the Supreme Court didn't intend for the Federal Circuit to extend *Mayo* as far as it has.

Like Judge Chen, Judge Moore agreed that diagnostic kits and techniques "are precisely the type of innovation the patent system exists to promote" and help to reduce healthcare costs through early disease detection. Diagnostic tests cost millions of dollars to develop and can be easily reproduced, making patent protection crucial.

> *Unless one opposes the notion of patent protection entirely, it cannot be reasonably disputed that claims to diagnostic kits and techniques, like pharmaceuticals, which require enormous initial investments in terms of both time and money, are the reason we suffer the promise of a monopoly.*

Judge Moore would put a finer point on the issue a few pages later:

> *The math is simple, you need not be an economist to get it: Without patent protection to recoup the enormous R&D cost, investment in diagnostic medicine will decline. To put it simply, this is bad. It is bad for the health of the American people and the health of the American economy. And it is avoidable depending on our interpretation of the Supreme Court's holding in Mayo.*

Moore reconciled *Mayo* with her belief that Athena's claims were patent eligible by noting that the claimed invention in *Mayo*, involving a relationship between the concentration of metabolites in the blood and dosage levels of thiopurine drugs, was not a new discovery of that relationship. By contrast, the claims in *Athena* involved the discovery of a type of antibody that binds to a particular receptor transmitting signals from neurons to muscles, leading to a completely new method for diagnosing a particular neurological disease that was capable of diagnosing about 20% of the patient population that had been previously undiagnosed. This was a new and useful discovery of a previously unknown relationship that should pass muster under Section 101, Moore opined.

Interestingly, even a cursory reading of Judge Moore's dissent reveals that her opinion was informed a great deal by the recent patent eligibility hearings held by the Senate Intellectual Property Subcommittee. Given that Moore was part of the Federal Circuit panel in this April's *Cleveland Clinic II* decision, which expressly rejected the notion that the U.S. Patent and Trademark Office's guidance on patent eligibility could impact the appellate court's case law, this would seem to underscore the need for Congressional action on Section 101 patent eligibility to give any weight to USPTO Director Iancu's efforts on subject matter eligibility.

Judge Newman's Dissent Joined by Judge Wallach

Judge Pauline Newman, the Federal Circuit's "great dissenter" who also issued a dissent in the *Athena* panel decision, wrote another one "because of the importance of medical diagnosis and the critical role of the patent system in achieving new diagnostic methods." Like Judge Moore, Newman said she believes that the Federal Circuit has "mistakenly enlarged" the Supreme Court's *Mayo* holding.

Despite the fact that the procedure claimed by Athena had not been previously used to diagnose *myasthenia gravis* (MG), Judge Newman found that the *Athena* panel majority's determination of ineligibility misapplied both the patent statute as well as *Mayo*. Rather than a law of nature, Athena's claims cover a new multi-step method of diagnosis and it was incorrect for the majority to omit the steps to perform the method from the claims. Whereas Judge Chen saw *Alice* as a reversion to *Flook*, Newman noted *Alice* reiterated that "an invention is not rendered ineligible for patent simply because it involves an abstract concept." Newman argued that statute and case law required that the claimed invention is considered as a whole. Viewed under correct law and precedent, Athena's claims meet Section 101's requirements and the appropriate patentability analysis should happen under Sections 102, 103 or 112.

Like Judge Moore, Judge Newman outlined several cases in which the Federal Circuit applied *Mayo* to invalidate claims covering methods of diagnosis. However, Newman further discusses several Federal Circuit cases in which methods of treatment have been found eligible under Section 101. These inconsistent rulings are incompatible with *Mayo*, which didn't create a Section 101 distinction between diagnostic methods and therapeutic methods. Newman also reviewed the concerns raised by *amici* in

this case regarding the Federal Circuit's Section 101 rulings and their effects on the development of diagnostic methods. She wrote:

> *This case presents an opportunity for judicial review and judicial remedy. Although diagnostic methods are not the only area in which section 101 jurisprudence warrants attention, Federal Circuit precedent is ripe for reconsideration specific to diagnostic methods, to correct our application of the Mayo decision and to restore the necessary economic incentive.*

Highlights From Other Concurrences and Dissents

Judge Lourie Concurring, Joined By Judges Reyna and Chen – "[W]e are bound by the Supreme Court's decision in *Mayo*… If I could write on a clean slate, I would write as an exception to patent eligibility, as respects natural laws, only claims directed to the natural law itself, e.g., $E=mc^2$, F=ma, Boyle's Law, Maxwell's Equations, etc. I would not exclude uses or detection of natural laws… But we do not write here on a clean slate…

"Amici and others have complained that our eligibility precedent is confused. However, our cases are consistent. They have distinguished between new method of treatment claims and unconventional laboratory techniques, on the one hand, and, on the other hand, diagnostic methods that consist of routine steps to observe the operation of a natural law, a clear line. Beyond that, I do not see a way clear to distinguish *Mayo* in a useful, principled, fashion."

Judge Hughes Concurring, Joined by Chief Judge Prost and Judge Taranto – "The multiple concurring and dissenting opinions regarding the denial of en banc rehearing in this case are illustrative of how fraught the issue of [Section] 101 eligibility, especially as applied to medical diagnostics patents, is… I, for one, would welcome further explication of eligibility standards in the area of diagnostics patents."

Judge Stoll Dissenting, Joined by Judge Wallach – "Federal Rule of Appellate Procedure 35 directs us to order rehearing en banc when 'the proceeding involves a question of exceptional importance…' [A] wholesale bar on patent eligibility for diagnostic claims has far-reaching and long-ranging implications for the development of life-saving diagnostic methods. The eligibility of life-saving inventions is not only one of the most important issues of patent law, but of human health. Thus, the importance of the issue here mandates that we consider it en banc."

Judge O'Malley Dissenting – "I write separately…because I believe that confusion and disagreements over patent eligibility have been engendered by the fact that the Supreme Court has ignored Congress's direction to the courts to apply [the Patent Act] as written. Specifically, the Supreme Court has instructed federal courts to read into Section 101 an 'inventive concept' requirement—a baffling standard that Congress removed when it amended the Patent Act in 1952…

"Because the Supreme Court judicially revived the invention requirement and continues to apply it despite express abrogation, I dissent to encourage Congress to clarify that there should be no such requirement read into [Section] 101; to clarify that concepts of novelty and 'invention' are to be assessed via application of other provisions of the Patent Act Congress designed for that purpose."