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July 2, 2010

Opposition Division
EUROPEAN PATENT OFFICE
27 Erhardtstr.
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Via fax and mail

Our ref.: MJF5718/SW/rg

Re: European Patent 1 831 699 B1
European patent application No. 05 820913.1
In the name of Antibodyshop A/S
Opponent: Abbott Laboratories

Dear Madams and Sirs,

this is to provide the Opponent's **requests, facts and arguments** with respect to the opposition lodged against the above-identified European patent (hereinafter referred to as the "Patent").

Extent of Opposition and the Requests

1. We request:
(i) the revocation of the European Patent No. 1 831 699 B1 in its entirety in all designated contracting states;
(ii) should the request under (i) not be granted in the written proceedings before the opposition division, oral proceedings in accordance with Article 116 EPC;
(iii) the issuance of a preliminary opinion in relation to the grounds raised in this opposition, at the time of the issuance of any summons to the oral proceedings, if deemed useful by the opposition division.

Grounds of Opposition

2. The Patent is opposed on the grounds set out under Article 100 EPC, more particularly Article 100(a), in particular for lack of novelty and lack of inventive step, Article 100(b), in particular that the Patent does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art, and Article 100(c), that the subject matter of the Patent extends beyond the content of the application as filed.

Documents relied on

3. European patent EP 1831699 B1 was filed on December 20, 2005 and claims a first priority date of December 20, 2004 and a second priority date of September 21, 2005. Therefore, everything that was made available to the public by means of a written or oral description, by use, or in any other way before December 20, 2004 is irrespective of the entitlement to priority, part of the state of the art under Article 54(2) EPC.

4. However, as is explained in paragraphs 14 – 20, it is submitted that none of the granted claims is entitled to the filing date of either of the priority documents and so can only take the filing date of December 20, 2005.

5. Accordingly, anything which was made available before December 20, 2005 is prior art under Article 54(2) EPC against any subject matter contained in EP 1 831 699 B1 – in particular contained in its granted claims – which has been stated on the filing date only, i.e., subject matter which is not entitled to the one or more of the preceding priority dates.

6. The following documents are relied on:

A1 : **Mishra, J. *et al.*** "*Neutrophil Gelatinase-Associated Lipocalin: A novel early urinary biomarker for Cisplatin Nephrotoxicity.*" Am. J. Nephrol.; **24**, 307-315. 2004 [Published online on May 12, 2004].

A2: **WO 2004/088276 A2** published on October 14, 2004 (= **D1** in Examination).

A3: **Mori K. *et al.*** *"Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury.*" The Journal of Clinical Investigation; **115**(3), 610-621, March 2005.

A4: **US 2005/0272101** published December 8, 2005 (counterpart WO 2005/121788 = **D4** in Examination).

A5: **Devarajan P.** "*Novel biomarkers for the early prediction of acute kidney injury.*" Cancer Therapy; **3**, 477-488, 2005.

A6: **Mehta R.L. *et al.*** "*Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury.*" Critical Care; **11** (2), R31, 2007.

A7: **Uttenthal, L.O.;** Renal Disease: *"NGAL: how useful is the new marker of kidney damage?"* Clinical Laboratory International, April 2007 [www.cli-online.com].

A8: **Xu S., Venge P.** "*Lipocalins as biochemical markers of disease.*" Biochim Biophys Acta; **1482** (1-2), 298–307, 2000.

A9: **Bangert K.** ***et al.*** *"NGAL is significantly increased in urine and plasma in acute renal failure."* Intensive Care Med.; **32** (Suppl. 1), S10, 2006.

A10: **Bewick V.** ***et al.***, *"Statistics review 13: Receiver operating characteristic curves."* Critical Care; **8**(6), 508-512, 2004.

A11: **Nielsen O.** ***et al.***, *"Rectal, Dialysate and Fecal Concentrations of Neutrophil Gelatinase-Associated Lipocalin, Interleukin-8,and Tumor Necrosis Factor-α in Ulcerative Colitis."* The American Journal Of Gastroenterology, **94** (10), 2923-2928, 1999 (= **D8** during Examination).

A12: **Dorland's Medical Dictionary, 29th Edition,** W.B. Saunders Company, New York (2000) [entries for *cancer, glomerulonephritis* and *neoplasm.*]

A13 **Wu** ***et al.*** "Analytical and clinical evaluation of new diagnostic tests for myocardial damage." Clinica Chimica Acta; **272**, 11-21 (1998).

A14 **Forsblad** ***et al.*** "Clinical manifestations of atherosclerosis in an elderly population are related to plasma neopterin, NGAL and endothelin-1, but not to Chlamydia pneumonia serology." Int. Angiol.; **21**(2): 173-9 (June 2002).

A15 **US 2003/175685 A1** published September 18, 2003.

A16 **Mori K.** ***et al.*** *"Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury."* The Journal of Clinical Investigation; **115**(3), 610-621, March 2005 – **Enlarged and magnified version of Figure 1C, with logarithmic y-axis values and gridlines added**.

Document **A6** is post-published and is introduced to explain the common general knowledge concerning technical terms at the filing date. Note that **A6** is accessible without charge via the internet at **http://ccforum.com/content/11/2/R31** and is flagged as a scientific article of high impact.

Document **A7** is also post-published and discloses some comments of Dr. Uttenthal, an inventor of the Patent and the CSO of Proprietor of the Patent, BioPorto[1], on the utility of NGAL as marker of renal damage.

Lastly, document **A9** (also by Dr. Uttenthal and extensively reviewed in **A7**) is

[1] AntibodyShop A/S changed its company name as of January 1, 2008 to BioPorto Diagnostics A/S, and since then uses the AntibodyShop name as a brand of BioPorto.

likewise post-published.

The Field Of The Claimed Invention

7. According to the Patent, paragraph [0001], the field of the claimed invention relates to:

methods for diagnosis and monitoring of human disease by means of measurement in a bodily fluid of human neutrophil gelatinase-associated lipocalin (NGAL), the abnormal concentration of which is indicative of a disease or group of diseases, in this instance disorders of the kidney resulting in decreased renal function, including those caused by ischemic injury (due to impaired blood supply to the kidney) or exposure to nephrotoxic agents or rejection of a transplanted kidney. The methods are particularly useful for the early detection of the renal response to ischemic injury, the clinical or pathologic consequences of which are typically acute renal failure (ARF), acute tubular necrosis (ATN) or acute tubulo-interstitial nephropathy (ATIN), and can also be used to monitor the course of renal disorders including the response to therapeutic measures.

The Independent Claim of the Patent

8. The Patent was granted with 17 claims, of which only claim 1 is independent. In the following, the lack of patentability of the granted claims will be discussed separately for each claim.

9. Claim 1 is directed to the following:

A method of diagnosing, monitoring or determining the likelihood of a renal disorder in a human being, wherein said method discriminates between a renal disorder and a condition that is not affecting the kidney, said method comprising the steps of

i) determining the concentration of human neutrophil gelatinase-associated lipocalin (NGAL) in a sample of bodily fluid from the human being,

ii) comparing said concentration with a predetermined cutoff value, said cutoff value being 250 ng/mL or a higher value, such as a value between 250 ng/mL and 525 ng/mL, chosen to exclude lower concentrations of NGAL associated with conditions that are not affecting the kidney, wherein a concentration above the cutoff value is indicative of a renal disorder.

Claim Interpretation

10. Claim 1 comprises two embodiments, a first broad one encompassing an open-ended range (*"a cutoff value being 250 ng/mL or a higher value"*) and a second, narrower one, drawn to a closed range (*"value between 250 ng/mL and 525 ng/mL"*).

11. The scope of the term renal disorder is broad. The Patent, page 4, paragraph [0007], lines 2 – 5 states:

"By renal disorder is meant any alteration of function, including the structural and ultrastructural correlates of that alteration, either of the kidney as a whole or of one or more cellular structures of which it is composed, that goes beyond the regulatory mechanisms which maintain the normal healthy state."

12. Specific renal disorders mentioned in paragraph [0007] include acute or chronic renal failure, as well as glomerulonephritis and neoplastic diseases of the kidney. See page 4, lines 2-9 of the Patent. Note that glomerulonephritis is an inflammatory disorder (e.g., as in Patent claim 4) and that neoplastic disease includes a cancerous disorder (e.g., as in Patent claim 6). See, e.g., **A12**.

13. As such, in Claim 1, "renal disorder" means *any* renal disorder and "a condition not affecting the kidney" means *any* condition *other than* a renal disorder.

Effective Date of the Claim

14. The claimed invention is not entitled to claim priority from either of the identified priority documents, US provisional application 60/637,503, filed 20 December 2004, or US provisional application 60/719,307, filed 21 September 2005.

15. Claim 1 calls for a predetermined cut off value of 250 ng/mL or higher; the only disclosure of a cut-off value in the first priority document is at page 12, lines 24-28 (discussion of **Table 1** results, in Example 4), which states:

"These results suggest that a cutoff value of about 1000 ng/mL (1 μg/mL) defines the urinary concentration of NGAL above which it becomes indicative of clinically significant renal affection. In an established case of ATN, the value is expected to be much higher."

16. This disclosure supports neither a predetermined cut off of 250 ng/mL, which is well below that of 1000 ng/mL, nor does this disclosure support a value "such as a value between 250 ng/mL and 525 ng/mL", the upper end of which is still below the only value mentioned in the first priority document.

As such, claim 1 is not entitled to claim priority from US 60/637503, the first priority filing.

None of the remaining claims refers to a higher cut off value. As such, none of the remaining claims is entitled to the first priority date.

17. In the second priority document, there are two disclosures of a cut off value. Page 7, lines 13-25 disclose:

"The cutoff level below which the urinary level of NGAL cannot be diagnostic of renal injury with an acceptable degree of specificity because such a level can be found in healthy individuals or those suffering from inflammatory,

infective or cancerous conditions is preferably a level between 0.5 μg/mL and 10 μg/mL, such as 1 μg/mL, or 1.5 μg/mL, or 2 μg/mL, or 3 μg/mL, or 4 μg/mL, or 5 μg/mL, or 6 μg/mL, or 7 μg/mL, or 8 μg/mL, or 9 μg/mL, or 10 μg/mL.

The cutoff level for the NGAL concentration in plasma or serum is preferably a level between 300 ng/mL and 900 ng/mL, such as 350 ng/mL, or 400 ng/mL, or 500 ng/mL, or 600 ng/mL, or 700 ng/mL, or 800 ng/mL, or 900 ng/mL."

18. Claim 2 of the second priority application discloses a cut off value in urine of 1 μg/mL or higher (i.e., 1000 ng/mL or higher, up to a recite value corresponding to 10,000 ng/mL or higher), and claim 3 discloses a cut off value for plasma and serum of 300 ng/mL or higher (up to a recited value of 900 ng/mL or higher), these preferences taken from page 7, lines 13-24 (see above).

19. As such claim 1 of the Patent is not entitled to claim priority from the second priority. As these ranges are not disclosed in any of the dependent claims, then none of the dependent claims are entitled to priority.

20. It follows that claim 1 of the Patent is only entitled to the filing date, 20 December 2005 of the international application. The same is true for the dependent claims.

ADDED MATTER (Article 123(2) EPC)

21. The Patent as granted contains subject matter which goes beyond that disclosed in the application as filed (as demonstrated by WO 2006/0666587) and as such contravenes Art 123(2) EPC.

22. Specifically, Claims 1-3 of the application as filed read:

1. A method of diagnosing, monitoring or determining the likelihood of a renal disorder in a human being, wherein said method discriminates between a renal disorder and another condition that does not affect the kidney, said method comprising the steps of

i) determining the concentration of human neutrophil gelatinase-associated lipocalin (NGAL) in a sample of bodily fluid from the human being,

ii) comparing said concentration with a predetermined cutoff value, said cutoff value being chosen to exclude lower concentrations of NGAL associated with conditions that do not affect the kidney, wherein a concentration above the cutoff value is indicative of a renal disorder.

2. The method of claim 1, wherein the sample is a urine sample and the cutoff value is 250 ng/mL or a higher value, such as a value between 250 ng/mL and 525 ng/mL.

3. The method of claim 1, wherein the sample is a plasma or serum sample and the cutoff value is 250 ng/mL or a higher value, such as a value between 250 ng/mL and 525 ng/mL.

[emphasis added to claims 1 - 3]

23. During prosecution of the application for the Patent, the proprietor amended claim 1 to incorporate the cut off values given in claims 2 and 3, so that claim 1 of the granted Patent reads:

1. A method of diagnosing, monitoring or determining the likelihood of a renal disorder in a human being, wherein said method discriminates between a renal disorder and a condition that is not affecting the kidney, said method comprising the steps of

i) determining the concentration of human neutrophil gelatinase-associated lipocalin (NGAL) in a sample of bodily fluid from the human being,

ii) comparing said concentration with a predetermined cutoff value, said cutoff value being 250 ng/mL or a higher value, such as a value between 250 ng/mL and 525 ng/mL, chosen to exclude lower concentrations of NGAL associated with conditions that are not affecting the kidney, wherein a concentration above the cutoff value is indicative of a renal disorder.

[emphasis added]

24. As the cut off values disclosed in claims 2 and 3 of the application as filed relate to urine and plasma or serum, respectively, if the scope of claim 1 in the Patent as granted goes beyond a bodily fluid selected from urine, plasma or serum, then matter will have been added. As no such limitation was made, claim 1 as granted contravenes Art .123(2) EPC and renders the Patent invalid.

INSUFFICIENCY OF DISCLOSURE (Article 83 EPC)

I. The invention cannot be performed over the whole range of the claim

25. Claim 1, in its first embodiment, requires that the cut off value is 250 ng/mL or higher. The cut off value is open ended. No upper limit is given for the cut off value in this embodiment and no guidance is given for its selection.

26. Using the methods described in **A10** (Bewick), sensitivity values can be determined from the data given in the Patent, **Table 2**, page 10-11. According to **A10**, page 508, left hand column:

*The **sensitivity** of a diagnostic test is the proportion of patients for whom the outcome is positive that are correctly identified by the test.*
*The **specificity** is the proportion of patients for whom the outcome is negative that are correctly identified by the test.*

27. Applying the methods of **A10**, the following sensitivities can be readily calculated from the data given by the Patentee at **Table 2** for urine and plasma, for cut off values >1000 ng/mL, >2000 ng/mL, >3000 ng/mL, >4000 ng/mL and > 5000 ng/mL. The results are set out in **Table A**:

Table A

Cut Off	Urine Values above the Cut Off	Urine Sensitivity	Plasma Values above the Cut Off	Plasma Sensitivity
>1000	24/34	0.71	18/34	0.53
>2000	21/34	0.62	4/34	0.12
>3000	17/34	0.50	2/34	0.05
>4000	11/34	0.32	0/34	0
>5000	0/34	0	0/34	0

28. **Table A** shows that considering urine samples, the sensitivity rapidly drops from 71% (24/34 patients) at >1000 ng/mL, to a wholly unacceptable 0% (i.e., every diagnosis would have been wrong!) at >5000 ng/mL. At >3000 ng/mL, the sensitivity is 50% (17/34) – i.e., random. Even worse results are obtained considering the plasma samples. Manifestly, the invention cannot be performed over the whole range claimed and so is not sufficiently disclosed.

II. The claimed invention cannot diagnose all kidney disorders

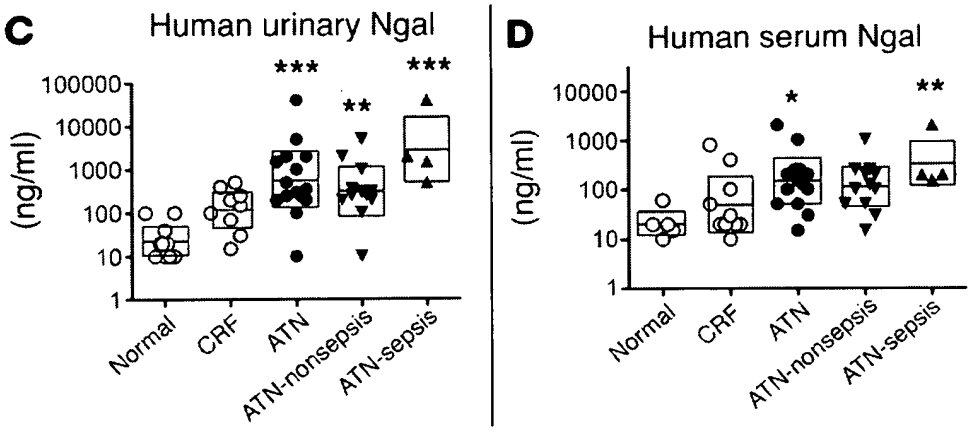
29. According to the Patent, page 4, paragraph [0007], line 6-7, renal disorder includes "chronic renal failure (CRF) of whatever cause. There is no evidence in the Patent to support the assertion in the Patent that the claimed method is able to distinguish CRF from other conditions not affecting the kidney, such as infection, in which NGAL levels are elevated. The data presented in **Table 2**, at page 10-12 does not distinguish between various types of "renal affection".

30. In contrast, **A3** (Mori *et al.,* 2005), right hand column, foot page 611 states that:

"... patients with chronic renal failure had less prominent elevations in serum Ngal (49 ng/ml; n = 10) and urinary Ngal (119 ng/ml; n = 9)"

31. Claim 1 requires a cut off of NGAL of at least 250 ng/mL in the sample of bodily fluid, which can be, for example, either urine or serum (see claims 2 and 3). This is well above the mean levels observed for CRF in **A3**, suggesting that the claimed method would generally NOT be capable of diagnosing CRF from a urine or serum sample using a cut off of at least 250 ng/mL.

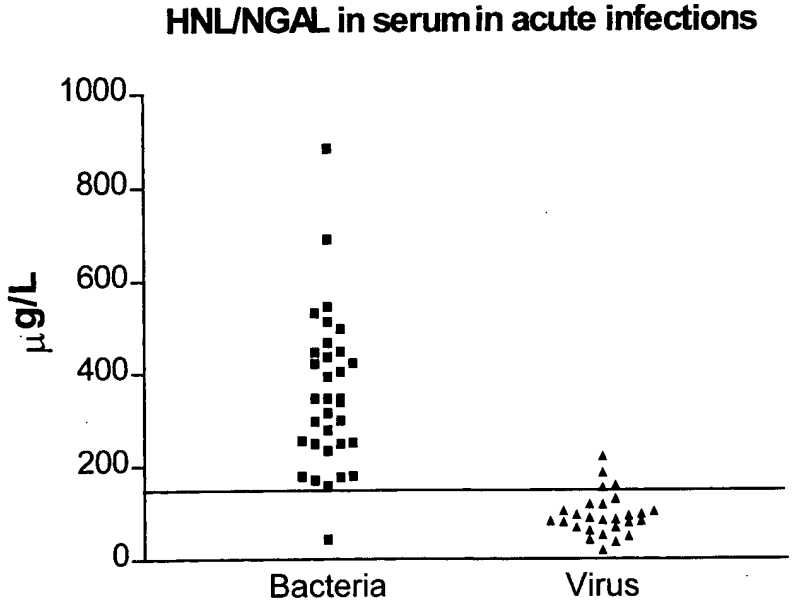
32. Further, in **A3**, page 611, Figure 1, individual datum points are given for patient urine samples, Figure 1(c) and serum samples, Figure 1(d) (reproduced below):



33. With a cut off of 250 ng/mL, only 3 out of 9 (33%) of the CRF patients would have been classified as having a kidney disease on the basis of the NGAL level in their urine samples, and 2 out of 10 (20%) would have been classified as having a kidney disease on the basis of their serum samples. This is far worse than the chance value of 50% and so the claimed method with a cut off level of 250 ng/mL cannot be used to diagnose for CRF. Since chronic renal failure is a renal disorder according to the Patent, claim 1 is invalid.

III. The claimed method is unable to distinguish between a kidney disorder and a bacterial infection

34. **A8** (Xu and Venge (2000)), page 304, quotes a study in which serum levels of NGAL distinguished between acute bacterial (elevated serum NGAL) and viral (normal serum NGAL). Figure 1 of **A8** is reproduced below.



35. These data confirm that with a cut off level of 250 ng/mL, an acute viral infection will not generally be diagnosed as a kidney disorder. However, of the 33 datum points given for patients diagnosed with bacterial infections, only 11 (33%) had serum NGAL levels below the 250 ng/mL cut off whereas 22

(67%) had NGAL levels above the cut off of the claim (19 of those, or 58%, within the range of 250 to 525 ng/mL), and would have been diagnosed as likely to have a kidney disorder. This again shows that the claimed invention is invalid.

IV. The claimed method is unable to distinguish between a kidney disorder and cancers

36. According to the Patent, the claimed invention discriminates between a renal disorder and a condition that is not affecting the kidney, such as cancerous disorders (see claim 6). Of the data presented in the Patent at **Table 2**, only four patients are classified as having cancer, but not a renal affection (select data from **Table 2** reproduced below as **Table B**). However, in all of the plasma measurements and three out of four of the urine measurements for these patients, NGAL levels were above the cut off of 250 ng/mL. Consequently, applying the method of the Patent, patients with cancer would have been mis-diagnosed as having a kidney disorder, rather than cancer.

Table B

Patient Number	Plasma NGAL Ng/mL	Urine NGAL ng/mL	Clinical classification			
			Renal Affection	Sepsis	Cancer	Hemodialysis
20	336	304	-	-	+	-
65	460	1705	-	-	+	-
80	322	328	-	+	+	-
100	354	85	-	-	+	-

37. Once again, the claimed invention is insufficient, as it is not able to discriminate between a renal disorder and another condition that is not affecting the kidney (cancer).

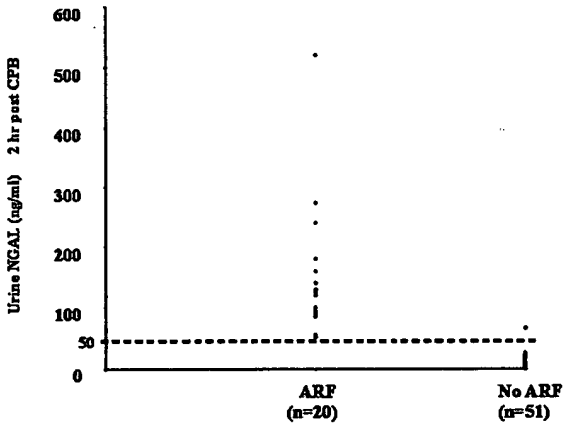
As such the claim is not operative across its entire scope and so is invalid.

V. The claimed method is unable to diagnose certain kidney disorders in a paediatric population

38. **A4** (US 2005/0272101, Devarajan) monitored the development of acute renal injury following cardiopulmonary bypass surgery in a paediatric population. By multivariate analysis, the amount of NGAL in urine at 2 hours after cardiopulmonary bypass was the most powerful independent predictor of acute renal injury. For concentration in urine of NGAL at 2 hours, the area under the receiver-operating characteristic (ROC) curve was 0.998, sensitivity was 1.00, and specificity was 0.98 for a cut off value of 50 ng/mL. For concentration in serum of NGAL at 2 hours, the area under the ROC curve was 0.906, sensitivity was 0.70, and specificity was 1.00 for a cut off value of 25 ng/mL. See **Table 2**, page 9.

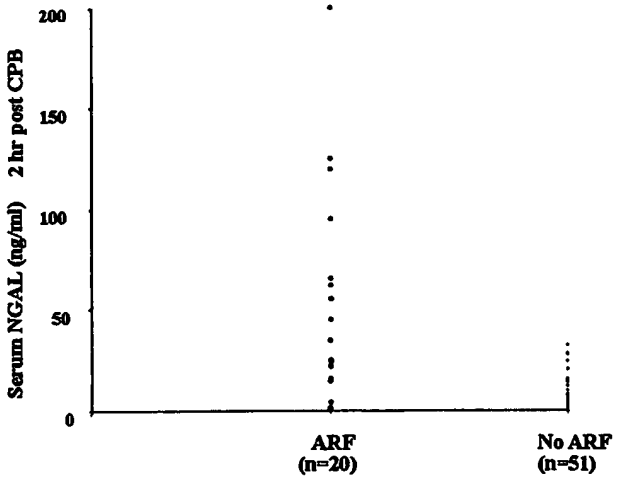
39. According to **A4** Figure 6 (reproduced below), although all 20 patients who developed an acute renal injury following surgery had urine NGAL levels 2 hours after surgery greater than the cut off value of 50 ng/mL, only 2 patients exceeded the 250 ng/mL cut off level required by the Patent claim. Based on this, applying the cut off in the Patent claims would have resulted in 18/20 of the patients being misdiagnosed as not having a renal disorder:

Figure 6



40. Similarly, in **A4** Figure 8 (reproduced below), none of the 20 patients who developed an acute renal injury following surgery had serum NGAL levels 2 hours after surgery exceeded the 250 ng/mL cut off level required by the claim. Therefore all of these patients would have been misdiagnosed as not having a renal disorder according to the method of the Patent.

Figure 8



41. In a review published in 2007 by Dr. Uttenthal, **A7**, this limitation of the method when applied to paediatric situations was recognised. Referring to a similar study to that of **A4** in **adult** cardiac surgery patients, Dr. Uttenthal states, page 2:

This study showed a marked rise in urinary NGAL even in the first post-operative sample that was taken. Interestingly, a notable rise was also seen in patients who did not subsequently develop a serum creatinine rise of 50% or more. However, in those patients in whom there was such a creatinine rise, the urinary NGAL level also continued to rise, peaking at three hours post-operatively, while the level fell in the other patients. ***The urine levels observed were such that any cutoff values diagnostic of subsequent acute renal dysfunction (ARD) at one hour or three hours after surgery would be many times greater than those observed in the paediatric cases*** *[reference omitted]*

[emphasis added]

42. In **A7**, Dr. Uttenthal notes under the heading "Special Cases" that cut off levels are very dependent on the patient populations:

"Urinary NGAL level has also been studied in children with diarrhea-associated haemolytic-uraemic syndrome [reference omitted]. Here a cutoff value of 200 ng/mL was found, and children with lower levels within the first five days of hospitalisation were much less likely to require dialysis. ***This provides further illustration of the fact that cutoff values for the optimal diagnostic or prognostic use of NGAL as a marker of kidney damage are very dependent on the patient population being studied.*** *"*

[emphasis added]

43. Under the heading "Chronic kidney disease", Dr. Uttenthal continues:

While the chief diagnostic role of NGAL is considered to be as a marker for acute renal injury, studies are also being carried out on its behaviour in chronic renal disorders. These include some very different pathologies, and it is evident that these will have very different effects on NGAL levels, and that the stage of the particular pathology will also be significant. ***It is therefore hardly to be expected that particular NGAL levels could be applied to any generalised diagnostic or prognostic evaluation of undifferentiated chronic renal failure.***

[emphasis added]

44. Dr. Uttenthal concludes, page 4:

If it were possible to measure NGAL exclusively of renal origin, it would probably be one of the best markers of renal tubule cell damage that could be conceived. In urine, NGAL shows a 10,000-fold concentration rise from normal levels in cases of renal injury; in plasma the maximum rise is about 100-fold. This makes NGAL potentially a very sensitive marker of different degrees of renal injury. However, the lower end of this wide dynamic range represents NGAL increases from extra-renal sources, so it will be the diagnostician's privilege and responsibility to take this into account.

45. As such even the inventor of the Patent seems to take the view that the scope of the diagnostic method is limited. No hint of these limitations is given in the claims nor is the skilled person taught by the Patent how to overcome these

limitations without undue experimentation or trial and error. For these reasons, the claimed invention fails to comply with Article 83 EPC and so is insufficient.

VI. There is no clinical definition of "renal disorder"

46. Claim 1 of the Patent allegedly provides a diagnostic method based on bodily fluid NGAL values able to discriminate a renal disorder from a condition which is not affecting the kidney. The Patent, page 4, paragraph [0007], lines 2-9 states that by renal disorder is meant:

"any alteration of function, including the structural and ultrastructural correlates of that alteration, either of the kidney as a whole or of one or more cellular structures of which it is composed, that goes beyond the regulatory mechanisms which maintain the normal healthy state. Non-exclusive examples of such disorders include conditions associated with renal ischemia such as acute tubular necrosis (ATN) or acute tubulo-interstitial nephropathy (ATIN), and also include acute renal failure (ARF) or chronic renal failure (CRF) of whatever cause, acute and chronic glomerulonephritis of whatever cause, nephropathy due to urinary obstruction, nephropathy due to hypertension, nephropathy associated with pre-eclampsia or toxemia of pregnancy, rejection or recurrent disease of a transplanted kidney, as well as congenital and neoplastic diseases of the kidney."

47. As discussed in paragraph 13 above, in Claim 1, "renal disorder" means *any* renal disorder and "a condition not affecting the kidney" means *any* condition *other than* a renal disorder.

48. According to the Patent, the discriminatory cut off values for NGAL are derived from correlations between measured urine NGAL values and clinical and paraclinical data, in particular from the plasma-creatinine level, presumably obtained at the time of measuring the reported NGAL level (see **Table 1**[2], pages 8-9) and from "discharge summaries and the results of routine blood tests (...) such as the plasma creatinine values" at an unspecified point in time (see **Table 2**, pages 10-12 and paragraph [0038]). However, **Table 2** does not report any plasma creatinine value actually measured.

49. According to **A5**, the classification of the sole condition of "acute renal failure" has been inconsistent in the past. See Chapters I and II, page 477 - page 478, first half of the left column of **A5**, stating *inter alia* (middle of left column at page 477) that:

"Although basic research and preclinical studies in animals have identified successful therapeutic interventions, translational clinical trials in humans have yielded disappointing results. One reason for this is the lack of a consensus operational definition for acute renal failure."

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[2] For patients 3 and 4 which do not have renal disorder according to Table 1, [0037] reports nevertheless about a hypothetical *later* rise in plasma-creatinine.

50. More particularly, the author of **A5** underscores in the passage bridging pages 477-478 with respect to ARF that

"Over 30 different definitions have been used in the clinical literature, ranging widely from minimal changes in serum creatinine (0.3 mg/dl or 20% increase above baseline) to severe ARF requiring dialysis (...) This lack of consensus definition, combined with the inherent shortcomings of serum creatinine measurements in ARF have seriously jeopardized the interpretation and comparison of existing clinical trials."

51. The fact that the diagnosis of acute renal failure has lacked, so far, a well-accepted common definition in terms of the serum creatinine levels involved, and that there is still an ongoing debate on this issue today is supported further by **A6**.

52. More particularly **A6** leaves no doubt that a universally-accepted definition of AKI – acute kidney injury, which is the new term replacing the older term ARF according to the new standards under development – did still not exist until after the Patent was filed. This is because **A6** describes an interim definition and staging system for AKI and a plan for further activities of the collaborative network which were developed at the first AKIN conference held in Amsterdam, The Netherlands, in September 2005, that is just before the filing date of the application for the Patent.

53. It goes without saying that under these circumstances, which show beyond the shadow of any doubt that **A5** is not an isolated disclosure, but that there was a stringent and widely recognized need in 2004-2005, for a reliable generic definition in the art (and for a respective staging system for fine-tuning purposes) the patent-in-suit -- which does not employ the term AKI -- should have provided, at the filing date, a definition of what was actually clinically intended by the term ARF, as employed in its specification.

54. However, this is not the case. On the contrary, by employing either the old term ARF, or even worse, the made up term "renal affection" (page 9, lines 15-17) no appreciable link to known external standards has been provided by the Patent.

55. Hence, the skilled person does not know which of the more than 30 known definitions mentioned in **A5** has been applied when reporting the results displayed in the tables of the patent-in-suit.

56. Incidentally, the team of Dr. Uttenthal felt the need to provide the particular definition of ARF relied upon in their studies somewhat later and lastly made it available upon presentation of the results to clinical practitioners – *after* the filing date of the Patent, see post-published document **A9**.

57. What is true for the sole ARF condition (see dependent claim 11) must be true all the more for the much broader species of "renal disorder" in general (e.g., claim 1). Thus, contrary to what is stated on page 9, lines 15-17, of the Patent:

"On the basis of discharge summaries and the result of routine blood tests, it was possible to classify (blindly with respect to NGAL data) 60 of these patients into those with and without a renal affection during their admission."

There is simply no standard definition of "renal disorder" in general in the scientific literature, and hence the criteria actually employed to decide, in Patent **Table 2**, what was renal affection, and what was not, are totally obscure.

VII. There is no clinical definition of particular renal disorders employed in the correlation with NGAL

58. As has already been noted, according to the Patent, at page 4, line 2 - line 9, the term "renal disorder" comprises a long list of kidney diseases ranging from chronic conditions over acute events to even neoplastic diseases of the kidney (see paragraph 12 above). However, for *none* of the elements of this long list, is a clinical definition given in the Patent, and hence again it is totally obscure for the skilled person, how the purported correlation with NGAL has been actually obtained for each of these diseases.

59. For example, the Patent does not teach how to discriminate between chronic renal disease and, e.g., non-renal cancer or sepsis. While the Patentee presented during prosecution in January 2008 an argument in Examination before the EPO that patients 51, 60, and 101 (plus two unreported patients) would have suffered from chronic renal disease at the time of running the study of **Table 2**, this was not perceivable by any means to a person skilled in the art from the application as originally filed.

60. Apparently, at least up to January 2008, any association with chronic renal failure was not even perceivable to the inventors themselves, see Dr. Uttenthal's comments in **A7** (paragraph 43 above).

61. While it would appear that inventors re-interpreted their results in 2008, hardly anyone could have foreseen, **in 2005**, why, how, and which results would have needed re-interpretation.

62. As it appears from the above, without the clinical definitions of (all) the renal disorders purportedly evaluated (and later correlated with NGAL) being given in the Patent, it is totally unclear to the skilled person as to which level of distinction between "renal disease" and "condition not affecting the kidney" the claimed cut off value actually relates.

VIII. Contradictory data in Tables 1 and 2 for certain renal disorders

63. Apart from the evident lack of enablement of the clinical definitions of the reported renal disorders, it would appear that, e.g., the link between a closed cut off level range of 250 to 525 ng/mL, and, e.g., ischemic injury (and its

related, downstream forms ARF, ATN and ATIN) is not disclosed in the patent-in-suit, contrary to Article 83 EPC.

64. The first priority application contained in its "Summary of the invention" a definition of kidney diseases confined to ARF, ATN and ATIN. The first priority application included **Table 1** and concluded that the values of **Table 1** suggest a cut off value of about 1000 ng/mL, with even higher values for confirmed ATN cases (see Patient No. 5 in **Table 1**). See in particular page 12, lines 24-28 of the first priority document.

65. Even though this conclusion was cancelled later in the non-provisional application as filed, the unchanged data disclosed in **Table 1** still suggested, on the filing date, the very same conclusion. This is because the conclusion is derived by numerical evaluation of the figures contained in the document.

66. Further, the Patent as granted mentions, as a remainder of the interpretation of the results displayed by **Table 1**, immediately after defining the closed range in paragraph [0017], that:

"In another example, the cutoff value used is a value of 1 microgram/ml or a higher value."

see page 5, lines 35-36 of the granted Patent. Thus, according to the disclosure of the Patent, a cut off value of 1000 ng/mL or higher applies to ischemic renal injury (ARF, ATN and ATIN), as shown by **Table 1**.

67. **Table 2**, on the other hand, does not make an individual break-down of the particular type of renal affection diagnosed for a given patient. Renal affection is totally undefined. Hence **Table 2** cannot be a basis for claiming specific cut offs for specific diseases.

68. The disclosure of the Patent (and of the application as originally-filed) therefore teaches unambiguously that the particular diseases recited in claims 10 and 11 are associated with a cut off value of 1000 ng/mL (or higher). Claims 10 and 11 as granted thus correctly depend from claim 1 only so long as the claim recites the open-ended range embodiment (cut off value of at least 250 ng/mL) as one possible alternative. Therefore, if the Proprietor, in response to the instant Opposition, were to confine claim 1 to the closed range embodiment alone (cut off value between 250 and 525 ng/mL), claims 10 and 11 would suddenly be associated with a much lower cut off value than originally disclosed in **Table 1**, and would contain new matter, contrary to Article 123(2) EPC.

69. In summary, for all the reason given above, Claim 1 and its dependent claims are insufficient and contrary to Article 83 EPC.

LACK OF NOVELTY (Art. 54 EPC)

70. The open-ended range embodiment of claim 1 lacks novelty over **A3** (Mori *et al.*) which was published before the second priority date, and hence is full prior art against claim 1. In **A3**, the expression of NGAL in acute tubular necrosis (ATN) of the human was investigated, see page 611, left column, under the heading "Results".

71. **A3** states that the urinary NGAL level of patients with ATN was found to be **557 ng/mL** (under "Results" at page 611, right column), thus falling plainly within the claimed range. Figures 1 A-D of **A3** display the outcome of human clinical tests, and the legend on top of the two columns at page 611 explains with respect to Figure 1A that:

"Human urine samples (0.1-20 µl) from patients with ATN showed high levels of NGAL, whereas samples from patients with chronic renal failure (CRF), patients with liver cirrhosis, hemochromatosis or pancreatic carcinoma but lacking a renal diagnosis (Others) or normal subjects (Normal) had low levels of NGAL."

See also Figure 1C displaying human urinary NGAL values for different patient groups.

72. It is clearly visible from Figures 1A and 1C of **A3** that patients undergoing ATN (acute tubular necrosis; cf. dependent claim 11 of the Patent) which is a renal disorder (cf. Patent page 4, line 24) had an increased NGAL signal in urine whose average was 557 ng/mL, as compared to normal patients, or compared to patients with, e.g., pancreatic carcinoma – i.e. individuals suffering from a condition not affecting the kidney. The Authors of **A3** conclude (page 611, end of right column) that "*these data correlate Ngal expression with acute kidney damage, implicating the kidney as the major source of serum and urinary NGAL*". In other words, NGAL bodily fluid values, elevated to the reported levels, are a fingerprint of acute kidney injury.

73. Hence, **A3** destroys the novelty of claim 1, to the extent that the open-ended range embodiment ("at least 250 ng/mL") is concerned.

74. Also the closed range embodiment of claim 1 lacks novelty over **A3** which was published before the second priority date, and hence is full prior art against claim 1.

75. This is because **A3** likewise discloses at page 611, Figure 1C, a cohort of ATN patients who do not suffer from sepsis. Like ATN in general, "ATN without sepsis" also qualifies as a "renal disorder" in the context of claim 1. As can be seen from Figure 1C, the mean value of this cohort amounts to about **310 ng/mL**. Namely, as is apparent from the enlarged and magnified version of Figure 1C enclosed herewith as **A16**, projection of the mean value graphically disclosed in **A3** onto the logarithmic y-axis provides a value comprised between 100 and 557 ng/mL, more in particular, about **310 ng/mL**.

76. Hence, **A3** also destroys the novelty of claim 1, to the extent that the closed range embodiment ("between 250 ng/mL and 525 ng/mL") is concerned.

LACK OF INVENTIVE STEP (Art 56 EPC)

I. Claim 1 cannot be carried out across its breadth.

77. The **open-ended** range embodiment of claim 1 lacks an inventive step in that it cannot be carried out across its breadth. Such a range comprises cut off values which are so high (e.g., like a cut off value of for example 10,000 ng/mL or even higher) that they do not discriminate over conditions not affecting the kidney. Actually none of the cases as per, for instance, Patent **Table 2** would be regarded as having renal affect if the cut off was chosen at this level, which nevertheless is covered by the Patent claim. Claim 1, contrary to prominent Decision T939/92, therefore cannot be carried out across its breadth because it clearly covers embodiments which lead to the wrong diagnosis, which is documented in the Patent itself. Claim 1 thus lacks inventive step.

II. Claim 1 covers trivial embodiments where no technical problem actually exists.

78. Due to its open language ("comprising" the steps of), claim 1 covers embodiments where the technical contribution of the cut off feature to the solution of the stated problem becomes non-critical, thus depriving claim 1 of any inventive step.

79. This applies to the carrying out of additional steps, e.g., the centrifugation of urine samples as per paragraphs [0015] and [0016], confirmed in the analytical methods of examples 1 and 2 of the Patent to pre-purify the sample from neutrophils. According to paragraph [0005] of the Patent, the latter are a major cause of false positives. Hence, where such centrifugation is actually carried out, there is no risk of interference by inflammation. Consequently little, if any, contribution of the cut off to the task of fading out false positives arises.

80. Similarly, as shown above at paragraphs 34 and 35, the claim encompass and yet the Patent disclosure does not provide, a method to distinguish between a kidney disorder and a bacterial infection. According to **A8** (Xu and Venge), using serum samples, there is no suggestion in the cited prior art suggesting that a bacterial infection interferes in the analysis of urine samples. Nor is there any reason to suppose that NGAL present in the blood as the result of a bacterial infection would give rise to NGAL in the urine. In any event, according to the data in **A8**, infections of viral origins would not interfere with a serum-based diagnosis of a renal disorder.

81. Furthermore, **A11** (Nielsen *et al.*), published in 1999, investigated the potential of NGAL as a disease activity marker for the inflammatory disease, ulcerative colitis, and concluded that not only did serum values of NGAL not mirror disease activity, but that in general, there was little difference from

control values for these other diseases. Of 76 data points, all but one (that at less than 250 ng/mL, i.e., about 180 ng/mL) had values of less than 100 ng/mL, irrespective of disease state. According to the Patent paragraph [0033], the concentration of NGAL in a pool of normal human plasma was 90 ng/mL.

82. Accordingly, it would seem that there is no technical problem to be solved in terms of an NGAL test discriminating between a renal disorder and a condition that is not affecting the kidney, in particular where that non-renal condition is an inflammatory disorder or an infective disorder. The prior art already showed that NGAL was an effective biomarker for kidney disease, particularly when using urine samples.

III. A4 as the Closest Prior Art

83. **A4** (US 2005/0272101) was published on 8 December 2005, that is before the filing date of the Patent. It discloses a method for the immediate or early onset detection of a renal tubular cell injury in a mammalian subject (see for example, **A4**, claim 1, reproduced below).

The method comprises the steps of
i.) determining the concentration of NGAL in a sample of body fluid (either urine or serum) from the human being
ii.) comparing said concentration with a predetermined cut off.

84. This method is exemplified at **A4**, page 9, Example 5, where it is stated, end of paragraph [0075]:

"For urine NGAL, a cut off of either 25 or 50 ng/ml yields outstanding sensitivity and specificity at both 2 hours and 4 hours post CPB. For serum NGAL at 2 hours CPB [cardiopulmonary bypass], sensitivity and specificity are optimal at the 25 ng/ml cut off."

85. The claimed invention differs from this closest prior art in that it relates to a different group -- those possibly suffering from a renal disorder -- and, there is a higher predetermined cut off, 250 ng/mL or higher, such as a value between 250 ng/mL and 525 ng/mL.

86. It would seem that the Patent term "renal disorder" is to be construed broadly, and includes post-ischemic renal injury (see claim 10), acute renal failure, acute tubular necrosis and acute tubulo-interstitial nephropathy (see claim 11) as well as a renal disorder caused by a nephrotoxic agent (claim 12), among others.

87. The main difference between the claimed invention and the closest prior art is in the higher predetermined cut off of:

"250 ng/mL or higher, such as a value between 250 ng/mL and 525 ng/mL".

III.a. The Problem to be Solved

88. The technical effect is that the diagnostic method determines the likelihood of renal disorder in a different patient group; the problem to be solved is that of finding a different patient group for which the diagnostic method could be used.

89. **A3** is concerned, in part, with the role of NGAL in acute tubular necrosis (ATN), which is one of the conditions specifically mentioned in the Patent as falling within the definition of a renal disorder. Although **A3** appears principally concerned with the treatment of renal disease, it would be consulted by the skilled person who is concerned with both the diagnosis and treatment of renal disorders. Indeed, both the inventors of **A4** (Devarajan and Barasch) are co-authors of **A3**.

90. As already noted, **A3**, page 611, legend to Figure 1, discloses that the concentration of NGAL in either urine or serum samples is able to discriminate between patients with acute renal failure and patients with liver cirrhosis, hemochromatosis, or pancreatic carcinoma but lacking a renal diagnosis.

91. **A3**, page 611, left column, under **Results**, *Expression of Ngal in ATN of the human,* discloses:

"Acute renal failure in humans was marked by log-order elevations in the concentration of serum and urinary Ngal protein. Compared with Ngal concentration in normal serum (21 ng/ml geometric mean, n = 5) and normal urine (22 ng/ml; n = 10), serum Ngal was elevated 7.3 fold (146 ng/ml; P<0.05, Fig 1) and urinary Ngal was elevated 25-fold (557 ng/ml; P<0.001) in our patients with ATN, the most typical form of acute renal failure."

92. **A3** continues, in the same paragraph:

"To determine whether Ngal expression correlated with the extent of acute renal impairment, we used simple regression analysis after log transformation of Ngal levels. We found that both serum Ngal (r =0.64, n = 32) and urinary Ngal (r = 0.68, n = 38), as well as Ngal normalized for urinary creatinine (r = 0.67, n = 36), were highly correlated with serum creatinine levels (P < 0.0001 each). In comparison, patients with chronic renal failure had less prominent elevations in serum Ngal (49 ng/ml; n = 10) and urinary Ngal (119 ng/ml; n = 9), and these values were not proportional to serum creatinine."

93. The skilled person is taught by **A3** that NGAL expression is correlated with the extent of acute renal impairment; that is the higher the NGAL level in either urine or serum, the more likely a patient is to be suffering from a renal disorder (in this case ATN). In particular, the skilled person is taught by **A3** to select a cut off value above the normal urine and serum NGAL concentrations (22 ng/mL and 21 ng/mL respectively) and below the mean level for patients with ATN (557 ng/mL for urine and 146 ng/mL for serum). If the skilled

person sought to further distinguish the renal impairment between chronic renal failure (CRF) and ATN, he would choose a cut off above the means determined for patients with CRF (119 ng/mL for urine, 49 ng/mL for serum) and below the means determined for patients with ATN. This would motivate the skilled person to select a cut off between 119 ng/mL and 557 ng/mL for urine for the diagnosis of ATN.

94. As already noted, sensitivity and selectivity values for different urinary cut offs can be readily determined from the data given in the Patent, **Table 2**, pages 10-11 using the method of **A10**, Bewick *et al.* (2004).

95. In the patient set examined for Patent **Table 2**, 34 of the 60 patients were diagnosed as having a renal affection, and 26 were diagnosed as not having a renal affection.

96. If a cut off of 25 ng/mL is set for the level above which NGAL is diagnostic of a renal affection, then 33 out of 34 patients (97%) would have been correctly diagnosed, giving a sensitivity of 0.97.

97. Similarly, at this cut off, 18 patients (out of 26) not having a renal affection would be wrongly diagnosed as having a kidney affection, leaving only 8 correctly identified as a negative outcome. This gives a specificity of 8/26, which is 0.31. Carrying out similar calculations for other cut off levels, using the proprietor's results in the Patent given at **Table 2**, we have determined the following sensitivity and specificities for a range of cut off values, reported below in **Table C**.

Table C

Urine NGAL ng/mL	*Renal Affection*	*No Affection*	*Sensitivity*	*Specificity*
> 0	*34*	*26*	*1*	*0*
>25	*33*	*18*	*0.97*	*0.31*
>50	*33*	*9*	*0.97*	*0.65*
>75	*33*	*6*	*0.97*	*0.80*
>100	*33*	*5*	*0.97*	*0.81*
>260	*32*	*5*	*0.94*	*0.81*
>525	*28*	*3*	*0.82*	*0.88*
Total	*34*	*26*		

98. According to the results given in the Patent, there is no significant difference in either sensitivity or specificity when taking an arbitrary cut off between urine levels of 75 ng/mL and 260 ng/mL.

As such there is no invention in selecting a urinary cut off greater than 250 ng/mL.

99. As far as the functional limitation of the claim, that the method discriminates between a renal disorder and a condition that is not affecting the kidney, such

as an inflammatory disorder (claim 4), an infective disorder (claim 5) or a cancerous disorder (claim 6), for the reasons give earlier, this also does not involve any inventive step.

100. In any event, from **A4**, page 1, paragraph [0007] it was already known that serum and urine NGAL levels are raised in bacterial but not viral infections. Thus the skilled person is already taught that viral infections do not pose a problem.

101. As such, the invention as claimed in claim 1, and the dependent claims, is devoid of inventive step and so contrary to Art 56EPC.

IV. Lack of inventive step over A3

102. The closed range embodiment of claim 1 (cut off between 250 and 525 ng/mL) also lacks – at least – an inventive step over **A3** (Mori). **A3** teaches, page 611, legend to Figure 1, that both urine and serum NGAL levels are able to discriminate between patients with acute renal failure and patients with liver cirrhosis, hemochromatosis or pancreatic carcinoma, but lacking a renal diagnosis. As outlined above, **A3** discloses a mean value of 557 ng/mL NGAL in urine of patients with ATN, the most typical form of acute renal failure.

103. Starting from **A3**, the objective technical problem would thus reside in providing, a different, alternative cut off value as distinctive feature in the diagnosis of ATN. In view of the small difference obtained (lower than 6% deviation to the upper limit of the claimed range) this problem was not solved by the Patentee.

104. Rather, taking into account that Patentee's values were obtained in a different hospital, the cut off value of **A3** was almost perfectly confirmed.

V. Lack of inventive step in the light of A4 starting from A1 as the closest prior art

105. **A1** states NGAL is a novel, early urinary biomarker for (cisplatin-induced) kidney damage (page 3, right column, end of the first paragraph). In the Introduction section of **A1**, the authors explain that earliness is material, as there is only a narrow window of opportunity for therapeutic measures, a window met by NGAL, but not by the "classic" marker serum creatinine. The Authors of **A1** conclude in the closing sentence on the last page, that all that is needed further is

"the establishment and validation of an ELISA procedure and eventually a point-of-care test."

106. The Patent, in the "detailed description of the invention", see in particular examples 1-6 appears to provide exactly these two – entirely expected – contributions.

107. Assuming, arguendo, that Patentee has actually done so, Patentee was however not the first. This is because the technical problem formulated in **A1** was only shortly later resolved by **A4**, addressing point-of-care kits in its paragraph [0033] and where the individual threshold values found are reported for several instances – see examples 4-6 of **A4**[3].

108. **A4** discloses *in extenso* how the correlation of the diagnostic results with the NGAL measurements is done and how the respective ROC curve is constructed – exactly like in the patent-in-suit. It therefore is not surprising that Mori (**A3**), and somewhat later also the Patentee, decided in view of **A4** to conduct similar investigations with patient populations not confined to children, and accordingly, in doing so, the Patentee and the team of Mori (**A3**) got similar, if not identical results.

VI. Lack of inventive step starting from A2 as closest prior art

109. **A2** teaches a method of detecting the early onset of renal tubular cell injury by measuring the biomarker NGAL. In particular, the reference teaches determining the concentration of NGAL in a mammal (including in a human) in urine in order to diagnose a renal tubular cell injury (Abstract and paragraphs [0037]-[0038], [0042]-[0044], [0062]). In addition, **A2** teaches the use of NGAL as a biomarker for monitoring the response to treatment (e.g., paragraphs [0038] and [0046]) and for predicting acute renal failure (ARF) (paragraph [0128]). With respect to the recitation that the method "discriminates between a renal disorder and another condition that does not affect the kidney," **A2** teaches that urine from patients with urinary tract infections contained only minimal quantities of NGAL, and was therefore "easily distinguishable" between the significantly greater quantities of NGAL observed in cadaveric kidney transplants (a model of ischemic renal injury). See Example 5. Consequently, the methods of **A2** are capable of discriminating renal tubular cell injuries from urinary tract infection, i.e., a condition that does not affect the kidney. Regarding the recitation that a concentration *above* the cut off level is indicative of a renal disorder, **A2** makes clear that increases in NGAL are indicative of renal disorder [0129].

110. It was well established background knowledge in the art at the time of the filing date of the purported invention to use cut off values in clinical assays as a means of comparison in order to objectively interpret laboratory test results. This is confirmed by **A10**, which teaches that the diagnostic accuracy of a test or assay -- i.e. the ability of the test or assay to distinguish between patients having a disease, condition or syndrome from those that do not -- is based on whether the patients have a clinically significant amount of an analyte higher than a predetermined cut off point or threshold value for that analyte. Changing this cut off point or threshold value usually changes the sensitivity and specificity of the diagnostic test. For example, if the threshold is lowered, sensitivity (true positive rate) will be increased while specificity (true negative rate) will be decreased. Similarly, raising the threshold will tend to decrease

[3] Inadvertently referred to as examples "4, 4 and 5" in **A4**.

sensitivity and increase specificity. This amounts to a trade-off between sensitivity and specificity: with greater sensitivity, there is a greater probability of correctly identifying the disease state, but at the cost of lower specificity which results in an increased occurrence of "false positives". Deciding which cut off is most appropriate depends on the goal of the screening procedure. If it is important to detect as many disease samples as possible, then setting the cut off at a point which maximizes sensitivity is warranted. By contrast, if false positives are undesirable, then setting the cut off at a point which maximizes the specificity is most appropriate.

111. **A13**, on the other hand, teaches certain criteria in selecting an appropriate cut off concentration (Abstract and Section 3.1). **A13** distinguishes between specific markers and nonspecific markers. As taught in Figure 1 of **A13** and the accompanying legend, highly specific markers enable the use of lower cut offs, while higher cut offs are used with nonspecific markers. For example, for specific markers, cut off concentrations that are at the upper limit of normal may be selected, whereas use of cut off concentrations higher than the upper limit may be necessary for nonspecific markers (page 15). The teachings of **A13**, which are evidently not confined to the specific marker observed by **A13**, therefore more generally indicate that those of skill in the art recognized that higher cut off values may be warranted when dealing with nonspecific markers.

112. In other words, the skilled person (who is aware of background knowledge as exemplified by **A10**), starting from **A2,** would recognize that NGAL may be present not only in the context of renal disorder, but also in the context of urinary tract infection, even though at lower levels. Thus, a skilled person, informed by the teachings of **A2**, and, after having read **A13**, would have had a reasonable expectation of success in selecting and using a cut off level that "discriminates between a renal disorder and another condition that does not affect the kidney." This is because **A2** taught that NGAL levels in patients with urinary tract infection were "easily distinguishable" from the significantly greater quantities of NGAL observed in cadaveric kidney transplants (a model of ischemic renal injury).

113. Thus, the subject matter of claim 1 is obvious over the combination of **A2** and **A13.** All that the proprietor did was to discover workable ranges, applying well known methods described in **A10,** and amounting to no more than routine experimentation.

114. How the experimentation and its interpretation were carried out is discussed in the following.

The Patent states that maximal urinary and plasma values were included in the ROC analysis. Using the data provided, the same analysis and calculations were performed for the urinary values:

Table D

	Patent Calculation Results (paragraph [0038]) **N = 60**	**Opponent's Calculation Results** **N = 60**
AUC	0.930	0.932
Sensitivity	0.969	0.912
Specificity	0.893	0.885
Positive Predictive Value	0.912	0.912
Negative Predictive Value	0.962	0.885
Cutoff	329-370 ng/mL	329-370 ng/mL

As seen in **Table D**, the results reported in the Patent cannot be replicated.

115. Instead, it can be seen that both values, sensitivity and specificity, especially the former, reported in the Patent to be associated with a cut off range of 329-370 ng/mL ([0038]) are higher than those obtained from Opponent's re-calculation based on the data in **Table 2**.

The fact that the sensitivity and specificity values are ***too high*** can be easily seen by comparing these values with those in **Table C** above (paragraph 97). **Table C** shows that a broader cut off range between 260 and 525 ng/mL will vary between 0.94 and 0.82 in sensitivity, and between 0.81 and 0.88 in specificity.

Evidently, the values reported by the Patentee (sensitivity 96.9% and specificity 89.3% for the smaller, "inner" range of 329 to 370 ng/mL) **do not fit into this picture** since both values, sensitivity and specificity, exceed any value encountered along the borders of the broader, "outer" range.

As can be seen from **Table C**, what is obtained, in reality, by shifting the cut off value beyond 260 ng/mL, is an improvement of specificity *to the detriment* of sensitivity – that is, to say, no more than the normal and expected trade-off typical for non-specific markers, as commented on in paragraphs 110 and 111, is produced.

116. The higher sensitivity/specificity values reported in the Patent appear to be due to a miscalculation by the Patentee.

In **Table 2**, n = 34 persons are labelled as having a renal affection.

However, the Patent states at paragraph [0040] that n = 32 (11+21) patients were included in the analysis for calculating the higher cut off value (i.e., the second cut off in claim 13 indicating need of dialysis) from the same **Table 2**.

Otherwise, using the data provided in **Table 2**, n = 34 patients are eligible to be included in this analysis. Using the data as it is provided in **Table 2**, the same analysis and calculations were performed as set out in **Table E** below:

Table E

	Patent Calculation Results **N = 32**	**Opponent's Calculation Results** **N = 34**
AUC	0.807	0.871
Sensitivity	0.870	0.800
Specificity	0.889	0.889
Positive Predictive Value	0.952	0.952
Negative Predictive Value	0.727	0.615
Cutoff	1338-2672 ng/mL	1338-2672 ng/mL

As seen from **Table E**, employing **Table 2** as disclosed leads to different results than reported in the Patent.

117. Thus, as per above, it was discovered that n = 32 patients were classified as having renal affect in the context of assessing the dialysis cut off value, even though n = 34 are labeled as such in **Table 2** of the Patent.
Using this information and the statistics provided for Figure 1 of the Patent, it was possible to back-calculate the statistics as presented in the Patent using a cutoff of 329 ng/mL. This back-calculation is illustrated in **Table F**:

Table F

	Renal Affect **+**	**No Renal Affect** **-**	
Above Cutoff (+)	*31* *(a)*	*3* *(b)*	*33* *(a + b)*
At/Below Cutoff (-)	*1* *(c)*	*25* *(d)*	*26* *(c +d)*
	32 **(a + c)**	28 (b + d)	**60** **(a + b+ c +d)**

- Since it is mentioned in [0040] that there are n = 32 people with "Renal Affect", this can be labeled (as per **Table F**) as the total number of "Renal Affect, +" , equivalent to $(a + c)$.

- Since there are n = 60 total persons $(a + b + c + d)$, the number of persons with "No Renal Affect, – " is equivalent to $(b + d)$, and must be 28.

- In paragraph [0038], a sensitivity of 0.969 is reported. Sensitivity is calculated as: SE = $a/(a + c)$. Using this formula and the fact that $(a + c) = 32$, the second cell of **Table F,** value (c), can be calculated. Namely, SE = 0.969 = $(a)/32$, therefore $(a) = 31$. Thus, $(c) = 1$ since $(a + c) = 32$.

- Furthermore, in paragraph [0038], a Positive Predictive Value of 0.912 is reported. Positive Predictive Value is calculated as: PPV = 0.912 = $(a)/(a + b)$. Using this formula and the fact that a = 31, cell (b) can be calculated. PPV = 0.912 = $31/(31 + b)$, so $(b) = 3$.

- Since cell $(b) = 3$ and $(b + d) = 28$, cell $(d) = 25$.

- With the rest of the cells filled in, it is easily confirmed that Specificity = $(d)/(b + d) = 25/28 = 0.893$ and Negative Predictive Value = $(d)/(c + d) = 25/26 = 0.962$, which are the values that are provided in paragraph [0038].

118. From the above back-calculation, the sensitivity and specificity values reported in the Patent for a cut off range of between 329 and 370 ng/mL have been confirmed. They are based on n = 32.

However, in order for there to be only n = 32 people with renal affect, n = 2 people must have been re-classified as not having renal affect since n = 34 persons are labeled has having renal affect in **Table 2** of the Patent.

Which patients were re-classified and *why* is not reported, however -- and it is even not *prima facie* perceivable that ***any patients at all*** were re-classified in arriving at the reported high sensitivity/specifity values associated in paragraph [0038] with the cut off range of 329-370 ng/mL.

119. Hence it appears, after calculating the cut off range based on n = 34 as reported in **Table 2** of the Patent, that all that the Patentee did in arriving at the "optimum" cut off range of 329-370 ng/mL was to increase specificity to the detriment of sensitivity. That is not only an obvious well-known option, but, also, as can be seen from **A13**, is the strategy of choice when using non-specific markers.

120. Moreover, **A13** does more than just indicate that whether a biomarker is specific or non-specific plays an important role in the selection of a cut off value, **A13** also documents the expectation that the normal reference range and cut off concentrations for any laboratory marker should be examined on an age- and sex-matched cohort group of healthy individuals (e.g., pages 14-16, Section 3.1).

121. **A14** measured NGAL levels in a population of older adults (median age 71 years). See, the Abstract; page 174, "Study population"; page 175, "Plasma NGAL and serum TNFR-1 analysis"; and page 177, left column. **A14** reports that in this "elderly" population, plasma levels of NGAL ranging from 53 to 263 ng/mL or from 67 to 241 ng/mL were observed (patients without or with

cardiovascular disease, respectively). See **Table 1**. Hence, since it was known that NGAL is normally elevated in bodily fluids of otherwise healthy elderly patients, in optimizing the cut off level one of ordinary skill in the art would have appreciated that the cut off level selected should be set at a level high enough to rule out such healthy patients in an age-matched cohort (i.e., diagnostic specificity considerations), while correctly identifying patients with even higher NGAL levels indicative of renal injury (i.e., diagnostic sensitivity considerations).

122. Moreover, in view of **A14**, it would have been obvious to the skilled person to arrive at the claimed invention by employing cut off values in plasma of, e.g., 263 ng/mL or higher when performing the methods of detecting renal injury of **A2.** Namely, one would be motivated to employ cut off values falling within the claimed ranges when detecting renal injury in elderly patients, for example.

VII. Summary of Inventive Step

123. In summary, claim 1 lacks an inventive step because it cannot be carried out across its breadth, because it covers trivial embodiments where no technical problem actually exists, because it is obvious starting from **A4** as the closest prior art, when read in the light of **A3**, because it lacks inventive step over **A3**, and because it lacks inventive step starting from **A1** when read in the light of **A4**. Moreover, claim 1 also lacks an inventive step starting from **A2** as closest prior art -- be it in the light of skilled person's background knowledge, and/or in the light of **A14.**

DEPENDENT CLAIMS

124. None of the dependent claims adds features or introduces limitations which overcome the objections raised above. In particular, we note the following.

Claim 2

125. Claim 2 focuses on urine samples. Since the above arguments focus inter alia on urine samples, claim 2 offends the EPC on the same grounds as specified above.

Claim 3

126. Claim 3 focuses on plasma or serum samples. The arguments put forward above, particularly, those under Article 83 EPC apply. In the event that the proprietor is able to convince the Opposition Division that the claimed invention is effective for plasma or serum samples, then, again for the reasons given above, it lacks inventive step over **A4** as the closest prior art in the light of the disclosure of **A3**.

Claim 4

127. Claim 4 focuses on inflammatory disorders as non-renal conditions. As stated above, the proprietor has failed to establish that there is a problem to solve in relation to an inflammatory disorder such as ulcerative colitis. As such there is no technical problem and so no inventive step.

Claim 5

128. Claim 5 focuses on infective disorders as non-renal conditions. However, as we have already pointed out, **A8** discloses that viral infections do not interfere with serum NGAL levels and so there is no technical problem to be solved. As far as bacterial infections are concerned, there is nothing to suggest that these interfere with NGAL levels in urine samples, so again there is no problem to be solved across the scope of the claim. To the extent that there is any problem to be solved, this is lacking in inventive step, as set out above, over **A4** as the closest prior art in the light of the disclosure of **A3**.

Claim 6

129. Claim 6 focuses on cancerous disorders as non-renal conditions. The legend to Figure 1A of **A3** (Mori) refers to pancreatic carcinoma as a non-renal condition from which ATN can be discriminated by NGAL levels in either urine or serum. Hence claim 6 offends the EPC on the same grounds as set out above.

Claims 7 and 8

130. Claim 7 concentrates on repeating the monitoring steps. **A4** discloses, for example Figure 2 and the legend thereto, the repeated monitoring, every two hours for a period of 12 hours of urine NGAL levels. As such there is no inventive step in selecting the features of either claims 7 or claim 8 and both claim 7 and 8 offend the EPC on the same grounds as set out above.

Claim 9

131. Claim 9 discloses to repeat the monitoring after treatment. **A4**, page 2, paragraph [0012] discloses a method of monitoring the effectiveness of a treatment for renal tubular injury which involves obtaining "at least one post treatment serum sample. In view of the earlier arguments based on **A4** and **A3**, this claim cannot therefore involve an inventive step. Hence claim 9 offends the EPC on the same grounds as set out above.

Claim 10

132. Claim 10 focuses on post-ischemic renal injury as renal condition. **A3** discloses, e.g. in the abstract that NGAL is highly accumulated in the blood and urine after ischemic injury. **A4** discloses, e.g., page 6, paragraph [0058] that NGAL is a sensitive, non-invasive serum biomarker for renal tubular cell

injuries, including renal ischemia. Hence claim 10 offends the EPC on the same grounds as set out above.

Claim 11

133. Claim 11 focuses on ARF, ATN or ATIN as consequences of renal disorder. **A4** is concerned throughout with acute renal failure (ARF), see e.g. paragraphs [0001], [0029] and [0058]. Mori (**A3**) refers to ATN in the legend to Figure 1. Hence claim 11 offends the EPC on the same grounds as set out above.

Claim 12

134. Claim 12 focuses on nephrotoxic agents as causes for renal failure. However it was already known at the date of filing that nephrotoxin cause NGAL to accumulate in the blood and the urine, see for example **A3**, abstract. Similarly, **A4** discloses, e.g., page 6, paragraph [0058] that NGAL is a sensitive, non-invasive serum biomarker for renal tubular cell injuries, including nephrotoxemia. Hence claim 12 offends the EPC on the same grounds as set out above.

Claim 13

135. Claim 13 covers the possibility to define NGAL-levels to be taken as triggering point of treatment. This is described in paragraph [0033] of **A4**, when referring to a "point-of-care" test. Hence claim 13 offends the EPC on the same grounds as set out above.

136. In addition to general detection of renal injury, **A2** also contemplates using its methods to estimate the *degree* of renal injury (e.g., paragraphs [0043] and [0046]). In particular, **A2** teaches that NGAL can be used to indicate the severity of renal injury by quantifying levels of this marker, since its excretion is proportional to the degree of injury (e.g., paragraphs [0127] to [0129]).

137. As the skilled person knows, severe grades of renal injury, in particular of ARF, generally require therapy by dialysis. **A15** also relates to clinical methods for diagnosing the presence of disease (in this case, liver fibrosis) by measuring the level of markers in samples (Abstract). **A15** contemplates the use of dual cut off values for the levels of the markers, in order to differentiate an absence of or mild disease on the one hand from moderate to severe disease on the other (see especially column 4, line 26 to column 5, line 52). In particular, a first cut off value is used in order to first rule out disease, followed by analysis of the "positive" population using a second cut off value to determine the presence of significant disease. See also column 14, line 37 to column 15, line 25; column 16, line 39 to column 17, line 55; and column 45, lines 10-21.

138. Hence, it would have been obvious to employ the known technique of **A15** of using a second cut off value to improve the similar methods of **A2** as discussed above at paragraphs 109-116, so that renal injury could be not only

detected but its severity assessed. Furthermore, in light of the common general knowledge that severe acute renal failure or severe renal injury were known to require treatment by hemodialysis, the ordinary artisan would have found it further obvious to conclude that patients determined to have more severe renal injury would be highly likely to require treatment by dialysis. Hence claim 13 lacks an inventive step over the combination of **A2** and **A15** in the light of common general knowledge.

Claim 14

139. Claim 14 covers a range of point-off care levels. The Patent, **Table 2** shows that 34 patients eligible to be included in the analysis were subsequently treated by hemodialysis. On the other hand, Figure 3 shows that only 32 patients were included in the analysis, which is confirmed by paragraph [0041] which refers to two groups of 11 and 21 patients, respectively. It is not apparent which patients were not included and why. Without this information, the analysis in the patent cannot be replicated. Hence claim 14 offends the EPC not only on the same grounds as set out above, but its subject matter is per se insufficiently disclosed, contrary to Article 83 EPC.

Claim 15

140. Claim 15 covers the use of NGAL-specific binding molecules. The antibodies employed by **A4** and **A3** certainly fall under this definition. Hence claim 14 offends the EPC on the same grounds as set out above.

Claim 16

141. This claim is a repetition of claim 2. Hence, it offends the EPC on the grounds mentioned earlier for claim 2.

Claim 17

142. This claim is an enlarged repetition of claim 3. Again, at least the arguments put forward against claim 3 under Article 83 EPC apply.

CONCLUSION

143. The above facts and arguments show that the subject matter claimed in the Patent does not meet the patentability requirements set forth under Articles 123(2), 54, 56 and 83 EPC.

The invention as claimed lacks novelty and inventive step, and the Patent includes added matter, and the invention is insufficiently disclosed.

As such the Patent should be revoked in its entirety for all designated member states.

In the event that the OD is minded to contemplate any other outcome, we confirm our request to be heard in Oral Proceedings.

Respectfully submitted

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