PATENT COOPERATION TREATY

TRANSLATION

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	See form PCT/ISA/210
Applicant's or agent's file reference: TM06Bü/Schö	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DE2004/002723	13.12.2004	23.12.2003

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/EP	Authorized officer
Facsimile No.	Telephone No.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/DE2004/002723

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rule 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☒ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☒ in written format

      ☒ in computer readable form

   c. time of filing/furnishing

      ☒ contained in the international application as filed.

      ☒ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/DE2004/002723

Box No. V **Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-10, 15-18	YES
	Claims	11-14	NO
Inventive step (IS)	Claims		YES
	Claims	1-18	NO
Industrial applicability (IA)	Claims	1-18	YES
	Claims		NO

2. Citations and explanations:

Reference is made to the following documents:

D1: SEYBOLDT C ET AL: "Reverse transcription-polymerase chain reaction assay for species-specific detection of bovine central nervous system tissue in meat and meat products." JOURNAL OF FOOD PROTECTION, vol. 66, no. 4, April 2003 (2003-04), pages 644-651, XP008048368 ISSN: 0362-028X

D2: LANGE BIANCA ET AL: "[Molecular biological detection of tissues of central nervous system in meat products]" BERLINER UND MUNCHENER TIERARZTLICHE WOCHENSCHRIFT. 2003 NOV-DEC, vol. 116, no. 11-12, November 2003 (2003-11), pages 467-473, XP008048367 ISSN: 0005-9366

D3: TANGA F Y ET AL: "Real time RT - PCR spinal assessment of the temporal regulation of glial activation and proinflammatory cytokines in a rat model of neuropathy." SOCIETY FOR NEUROSCIENCE ABSTRACT VIEWER AND ITINERARY PLANNER, vol. 2003, 2003, pages abstract no. 696.19 URL-http://sf, XP008048399 & 33RD ANNUAL MEETING OF THE SOCIETY OF NEUROSCIENCE; NEW ORLEANS, LA, USA; NOVEMBER

08-12, 2003

D4: BECKER ALBERT J ET AL: "Transcriptional profiling in human epilepsy: expression array and single cell real-time qRT-PCT analysis reveal distinct cellular gene regulation." NEUROREPORT. 19 July 2002, vol. 13, no. 10 19 July 2002 (2002-07-19), pages 1327-1333, XP008048414 ISSN: 0959-4965

1. The present application does not meet the requirements of **PCT Article 33(1)** because the subject matter of **claims 11-14** is not novel under **PCT Article 33(2)**, see documents **D1** and **D2** in this respect.

2. The present application does not meet the requirements of **PCT Article 33(1)** because the subject matter of **claims 1-18** does not involve an inventive step under **PCT Article 33(3)**.

2.1 **D1** is considered the **closest prior art** to the subject matter of **claim 1**. It discloses the development of an RT-PCR method for specific detection of glial fibrillary acidic protein (GFAP) to detect contaminations of central nervous system tissue in meat products. This method is a species-specific method which explicitly is also applicable to homogenized and heat-treated meat or meat products. The authors of **D1** are able to detect the GFAP transcript of the species cattle, pig, sheep etc. by using the RT-PCR method set forth herein. The sample material taken is prepared by homogenization and RNA is obtained by the method according to Chomczyinski & Sacci, i.e. by classical phenol-based work-up, see the whole

document **D1**.

2.2 The subject matter of **claim 1** therefore differs from the closest prior art (**D1**) in that a real time PCR [*eine PCR in Echtzeit*] is carried out which is intended to enable the GFAP transcript to be evaluated accurately and quantitatively.

2.3 The problem addressed by the present invention can therefore be considered that of providing a method for quantitative determination of the GFAP transcript [*quantitatives Bestimmungsverfahren für das GFAP Transkript*] which method may be employed in order to detect contaminations of CNS tissue in meat/meat products.

2.4 This problem is solved by the applicant providing a real time PCR for amplification and quantitative detection of the GFAP transcript.

2.5 The solution proposed in claim 1 of the present application **cannot be considered inventive (PCT Article 33(3))**, for the following reasons:

2.5.1 Amplification of the GFAP transcript in real time has already been disclosed in the prior art. **D3** provides a real time RT PCR for the GFAP transcript. **D4** provides quantitative single-cell real time RT PCR for the GFAP transcript.

2.5.2 The use of quantitative real time RT PCRs for the transcript of the GFAP gene has been disclosed in the

prior art. A person skilled in the art who would like to detect said transcript of the GFAP gene not only qualitatively but also **quantitatively** in order to detect possible central nervous system tissue contaminations in meat products would use for this purpose a quantitative RT PCR of the prior art rather than a qualitative RT PCR. In summary, the feature "quantitative real time RT PCR" is only one of several obvious possibilities from which a person skilled in the art would choose according to the circumstances in order to solve the problem of interest, without thereby being inventive.

2.5.3 The dependent claims contain, as additional features to the prior art, merely primer specificities which are intended to be used for detecting the GFAP transcript. Since the GFAP gene sequence data of different species are known, see in this respect the list of references of the present application and **D1** and **D2** of the prior art, developing amplification primers must be regarded as being molecular-biological routine procedure which proceeds with certain well-known parameters being observed (similar melting points, no formation of secondary structures, avoiding primer dimer structures etc.) and without inventive input. The primers provided in the present application in order to amplify the GFAP gene could be considered inventive only if said primers had unexpected effects or properties which would be delimiting over the prior art. However, the present selection is only one of several obvious possibilities from which a person skilled in the art would choose according to the circumstances in order to solve the problem of interest, without thereby being inventive.

Box No. V **Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

2.6 The present application does not meet the requirements of **PCT Article 33(1)** because the subject matter of **claims 1-18** does not involve an inventive step under **PCT Article 33(3).**