

## Claims

1. A method for determining the presence or absence of one or more specific serogroups of pathogenic Shiga toxin-producing *E. coli* (STEC) in a food sample, comprising
  - incubating the food sample in a culture medium enabling growth of *E. coli* to obtain an *E. coli* stock medium;
  - performing a lysis of the *E. coli* in the stock medium to obtain a sample solution comprising *E. coli* DNA; and
  - subjecting the sample solution to a first series of polymerase chain reactions (PCR), using primers to amplify the following *E. coli* genes or fragments thereof:
    - *stx1* and/or *stx2* encoding Shiga toxin 1 and Shiga toxin 2, respectively,
    - for each STEC serogroup to be determined, the subtype of the *eae* gene of that serogroup encoding an intimin, and
    - for each STEC serogroup to be determined, a biomarker gene specific for that serogroup;and, in case that *stx1* and/or *stx2* as well as the *eae* gene and the specific biomarker gene for at least one serogroup are amplified in the first series of PCRs:
  - verifying the presence of the one or more specific serogroups of STEC by performing a second series of PCRs on concentrates of the *E. coli* stock medium and, optionally, a third series of PCRs on single *E. coli* colonies derived from the concentrates.
2. The method of claim 1, wherein the verification of the presence of the one or more specific serogroups of STEC comprises
  - performing an immunoconcentration of the *E. coli* stock medium for each serogroup, using antibodies directed against the STEC of the respective serogroup;
  - isolating DNA from the immunoconcentrates; and
  - subjecting the DNA to a second series of PCRs, using primers to amplify the following *E. coli* genes or fragments thereof:

- *stx1* and/or *stx2*,
  - the subtypes of the *eae* gene of the respective serogroups, and
  - optionally, the biomarker genes specific for the respective serogroups.
3. The method of claim 2, wherein the verification of the presence of the one or more specific serogroups of STEC further comprises, in case that *stx1* and/or *stx2* as well as the *eae* genes are amplified in the second series of PCRs:
- plating the immunoconcentrates on a chromogenic medium to enable growth of isolated *E. coli* colonies;
  - isolating DNA from the isolated colonies; and
  - subjecting the DNA to a third series of PCRs, using primers to amplify the following *E. coli* genes or fragments thereof:
    - *stx1* and/or *stx2*,
    - the subtypes of the *eae* gene of the respective serogroups, and
    - optionally, the biomarker genes specific for the respective serogroups.
4. The method of any one of the preceding claims, wherein the series of PCRs are performed with specific labeled DNA probes to allow a detection of the amplified genes or fragments.
5. The method of any one of the preceding claims, wherein the specific serogroup(s) to be determined are selected from O157, O26, O45, O103, O111, O121 and O145.
6. The method of claim 5, comprising the determination of the presence or absence of all serogroups O157, O26, O45, O103, O111, O121 and O145.
7. The method of claim 5 or claim 6, wherein the subtypes of the *eae* gene to be amplified in the series of PCRs are selected from *eae*  $\beta$  for O26,

*eae*  $\gamma$  for O145 and O157, *eae*  $\epsilon$  for O45, O103 and O121, and *eae*  $\theta$  for O111.

8. The method of any one of the preceding claims, wherein the specific biomarker genes are selected from the O-antigen transporter genes of the respective serogroups.
9. The method of any one of the preceding claims, wherein the food sample is selected from raw meat, in particular raw beef meat, a raw dairy product, or a raw vegetable product, in particular a sprout.
10. The method of any one of the preceding claims, wherein the culture medium for the incubation step is selected from buffered peptone water, optionally comprising acriflavine; tryptic soy broth (TSB), optionally comprising novobiocine; and modified tryptic soy broth (mTSB), optionally comprising novobiocine.
11. The method of any one of the preceding claims, wherein the PCRs of each series are performed simultaneously in an automated manner.
12. The method of any one of claims 3 to 11, wherein the chromogenic medium is selected from Cefixime-Tellurite Sorbitol McConkey (CT-SMAC); modified Rainbow Agar (mRBA) comprising novobiocine, cefixime trihydrate and potassium tellurite; Tryptone Bile X Glucuronide medium (TBX); and Cefixime-Tellurite Rhamnose McConkey (CT-RMAC).
13. A reaction cartridge for performing a series of PCRs on a DNA-containing sample solution comprising a plurality of reaction chambers and a reservoir from which the sample solution can be homogeneously distributed into the reaction chambers, wherein at least some of the reaction chambers are preloaded with primers and probes to amplify the following *E. coli* genes or fragments thereof:
  - *stx1* and/or *stx2*, and
  - the subtypes of the *eae* gene of one or more serogroups selected from O157, O26, O45, O103, O111, O121 and O145.

14. The reaction cartridge of claim 13, wherein further at least some of the reaction chambers are preloaded with primers and probes to amplify the following *E. coli* genes or fragments thereof:
- biomarker genes specific for one or more serogroups selected from O157, O26, O45, O103, O111, O121 and O145.
15. An apparatus for automatically performing a series of PCRs, wherein the apparatus is adapted to accommodate the reaction cartridge of claim 13 or 14, and wherein the apparatus comprises
- detection means for detecting the amplified *E. coli* genes with the aid of the respective probes;
  - control means for controlling the performance of the PCRs, wherein a software is stored in the control means which is able to evaluate the detected *E. coli* genes according to an algorithm, such that a final result is generated for each serogroup depending on the detection results for *stx1* and/or *stx2*, for the subtypes of the *eae* gene of that serogroup, and optionally for the biomarker gene specific for that serogroup; and
  - indicator means for optically and/or acoustically indicating the final result for each serogroup.

## Claims

1. A method for determining the presence or absence of one or more specific serogroups of pathogenic Shiga toxin-producing *E. coli* (STEC) in a food sample, comprising
  - incubating the food sample in a culture medium enabling growth of *E. coli* to obtain an *E. coli* stock medium;
  - performing a lysis of the *E. coli* in the stock medium to obtain a sample solution comprising *E. coli* DNA; and
  - subjecting the sample solution to a first series of polymerase chain reactions (PCR), using primers to amplify the following *E. coli* genes or fragments thereof:
    - *stx1* and/or *stx2* encoding Shiga toxin 1 and Shiga toxin 2, respectively,
    - for each STEC serogroup to be determined, the subtype of the *eae* gene of that serogroup encoding an intimin, and
    - for each STEC serogroup to be determined, a biomarker gene specific for that serogroup;

and, in case that *stx1* and/or *stx2* as well as the *eae* gene and the specific biomarker gene for at least one serogroup are amplified in the first series of PCRs:

- verifying the presence of the one or more specific serogroups of STEC by performing a second series of PCRs on concentrates of the *E. coli* stock medium and, optionally, a third series of PCRs on single *E. coli* colonies derived from the concentrates,

wherein the verification of the presence of the one or more specific serogroups of STEC comprises

- performing an immunoconcentration of the *E. coli* stock medium for each serogroup, using antibodies directed against the STEC of the respective serogroup;
- isolating DNA from the immunoconcentrates; and
- subjecting the DNA to the second series of PCRs, using primers to amplify the following *E. coli* genes or fragments thereof:

- *stx1* and/or *stx2*,
  - the subtypes of the *eae* gene of the respective serogroups, and
  - optionally, the biomarker genes specific for the respective serogroups.
2. The method of claim 1, wherein the verification of the presence of the one or more specific serogroups of STEC further comprises, in case that *stx1* and/or *stx2* as well as the *eae* genes are amplified in the second series of PCRs:
- plating the immunoconcentrates on a chromogenic medium to enable growth of isolated *E. coli* colonies;
  - isolating DNA from the isolated colonies; and
  - subjecting the DNA to a third series of PCRs, using primers to amplify the following *E. coli* genes or fragments thereof:
    - *stx1* and/or *stx2*,
    - the subtypes of the *eae* gene of the respective serogroups, and
    - optionally, the biomarker genes specific for the respective serogroups.
3. The method of any one of the preceding claims, wherein the series of PCRs are performed with specific labeled DNA probes to allow a detection of the amplified genes or fragments.
4. The method of any one of the preceding claims, wherein the specific serogroup(s) to be determined are selected from O157, O26, O45, O103, O111, O121 and O145.
5. The method of claim 4, comprising the determination of the presence or absence of all serogroups O157, O26, O45, O103, O111, O121 and O145.

6. The method of claim 4 or claim 5, wherein the subtypes of the *eae* gene to be amplified in the series of PCRs are selected from *eae*  $\beta$  for O26, *eae*  $\gamma$  for O145 and O157, *eae*  $\varepsilon$  for O45, O103 and O121, and *eae*  $\theta$  for O111.
7. The method of any one of the preceding claims, wherein the specific biomarker genes are selected from the O-antigen transporter genes of the respective serogroups.
8. The method of any one of the preceding claims, wherein the food sample is selected from raw meat, in particular raw beef meat, a raw dairy product, or a raw vegetable product, in particular a sprout.
9. The method of any one of the preceding claims, wherein the culture medium for the incubation step is selected from buffered peptone water, optionally comprising acriflavine; tryptic soy broth (TSB), optionally comprising novobiocine; and modified tryptic soy broth (mTSB), optionally comprising novobiocine.
10. The method of any one of the preceding claims, wherein the PCRs of each series are performed simultaneously in an automated manner.
11. The method of claim 2, wherein the chromogenic medium is selected from Cefixime-Tellurite Sorbitol McConkey (CT-SMAC); modified Rainbow Agar (mRBA) comprising novobiocine, cefixime trihydrate and potassium tellurite; Tryptone Bile X Glucuronide medium (TBX); and Cefixime-Tellurite Rhamnose McConkey (CT-RMAC).