

CLAIMS

1. An *in vitro* method for predicting the chronological age of healthy *Galliformes*, the method comprising the steps of:
- 5 (a.) obtaining genomic DNA from biological sample material deriving from the *Galliformes* subject or from the *Galliformes* population to be tested,
- (b.) determining the methylation level of a set of specific CpG sites in the genomic *Galliformes* DNA obtained in step (a.), and
- 10 (c.) comparing the methylation levels of these CpG sites in the genomic *Galliformes* DNA from the sample to be tested with the methylation level of the same CpG sites from an age-correlated reference sample,
- thereby establishing the epigenetic age and predicting the chronological age of the subject or of the population to be tested;
- 15 the method being **characterized in that** for the set of specific CpG sites in step (b)
- the impact of genetic polymorphisms is eliminated by excluding CpG sites associated with single nucleotide polymorphisms, and
 - the impact of sex-specific methylation differences on sex chromosomes is eliminated by excluding all CpG sites located on sex chromosomes.
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2. The method according to claim 1, **characterized in that** the methylation levels of the set of the specific CpG sites in step (b) were normalized tissue-specifically.
3. The method according to claim 1 or according to claim 2, **characterized in that** the
- 25 *Galliformes* subject or population to be tested belongs to the species *Gallus gallus* and the set of specific CpG sites comprises or consists of the CpG sites indicated in Table 1.
4. The method according to claim 1 or according to claim 2, **characterized in that** the
- Galliformes* subject or population to be tested belongs to the species *Gallus gallus* and the set of
- 30 specific CpG sites comprises or consists of the CpG sites indicated in Table 2.
5. The method according to claim 1 or according to claim 2, **characterized in that** the
- Galliformes* subject or population to be tested belongs to the species *Gallus gallus* and the set of specific CpG sites comprises or consists of the CpG sites indicated in Table 3.
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6. The method according to any one of the preceding claims, **characterized in that** the biological sample material deriving from the subject or from the population to be tested is selected from the group consisting of body fluids, excremental material, tissue material, such as muscle tissue, organ tissue, such as gut tissue, skin tissue, feather material, or combinations thereof.
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7. The method according to any one of the preceding claims, **characterized in that** step (b.) includes a DNA methylation profiling process, preferably bisulfite sequencing.
8. The method according to any one of the preceding claims, **characterized in that** the
5 *Galliformes* subject or population to be tested is/are broiler(s) having a life span of up to 63 days.
9. An *in vitro* method for estimating the inflammation status in *Galliformes*, the method comprising the steps of:
(a.) obtaining genomic DNA from biological sample material deriving from the *Galliformes* subject or
10 from the *Galliformes* population to be tested,
(b.) determining the methylation level of a set of specific CpG sites in the genomic *Galliformes* DNA obtained in step (a.), and
(c.) comparing the methylation levels of these CpG sites in the genomic *Galliformes* DNA from the sample to be tested with the methylation level of the same CpG sites from an age-correlated
15 reference sample,
thereby establishing the epigenetic age of the subject or of the population to be tested, and
(d.) comparing the thus-obtained epigenetic age of the subject or of the population to be tested with its actual chronological age,
wherein an epigenetic age higher than the chronological age is indicative of inflammation,
20 the method being **characterized in that** for the set of specific CpG sites in step (b)
- the impact of genetic polymorphisms is eliminated by excluding CpG sites associated with single nucleotide polymorphisms, and
- the impact of sex-specific methylation differences on sex chromosomes is eliminated by excluding all CpG sites located on sex chromosomes.
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10. The method according to claim 9, **characterized in that** the methylation levels of the set of the specific CpG sites in step (b) were normalized tissue-specifically.
11. The method according to claim 9 or according to claim 10, **characterized in that** the
30 *Galliformes* subject or population to be tested belongs to the species *Gallus gallus* and the set of specific CpG sites comprises or consists of the CpG sites indicated in Table 1.
12. The method according to claim 9 or according to claim 10, **characterized in that** the
Galliformes subject or population to be tested belongs to the species *Gallus gallus* and the set of
35 specific CpG sites comprises or consists of the CpG sites indicated in Table 2.
13. The method according to any one of claims 9 to 12, **characterized in that** the biological sample material deriving from the subject or from the population to be tested is selected from the group consisting of body fluids, excremental material, tissue material, such as muscle tissue, organ
40 tissue, such as gut tissue, skin tissue, feather material, or combinations thereof.

14. The method according to any one of claims 9 to 13, **characterized in that** step (b.) includes a DNA methylation profiling process, preferably bisulfite sequencing.
- 5 15. The method according to any one of claims 9 to 14, **characterized in that** the *Galliformes* subject or population to be tested is/are broiler(s) having a life span of up to 63 days.