**Supporting Information**

**Supporting Methods**

Study familiarization was conducted before the main trial to ensure that the sites were acquainted with the PIVKA-II and AFP reagents and the electronic data capture system. Before each experimental run, the external study sites were requested to run a daily quality control on all systems and measuring cells in use on the experimental day, and to proceed only if these were within the target range of the assay (i.e. ± 3 standard deviations of the mean).

Relevant patient information was obtained for all participants entered into the study and recorded on the appropriate case report form (paper or electronic). Demographic information (sex, age and race), date of informed consent, specimen information (draw date, matrix type, appearance and storage before shipment to the testing sites), and clinical information (history of diagnosis, therapy, and medical history of other disease) were requested for all participants. All clinical information was collected in an electronic data capture system.

Frozen serum samples were shipped from collection sites to Roche on dry ice in a thermally insulated container. Good Laboratory Practices and national regulations for shipping of samples were followed. Samples were received at the destination in a frozen state and immediately stored at -70 °C or below until testing. The same procedure was followed during shipment of samples from Roche to testing sites.

The sample size for the study was determined based on bootstrapped simulation analysis to evaluate whether a lower number of samples could be used for the primary analysis of clinical endpoints. This revealed that at least 150 HCC cases and 150 controls were required to assure greater than 80% statistical power.

# Table S1 Demographic characteristics of study participants

|  |  |  |  |
| --- | --- | --- | --- |
|  | **All (*N* = 376)** | **Control (*n* = 208)** | **HCC (*n* = 168)** |
| Mean ± SD age (years) | 56.95 ± 12.42 | 52.18 ± 12.27 | 62.86 ± 9.82 |
| Male, *n* (%) | 267 (71.0) | 126 (60.6) | 141 (83.9) |
| Race, *n* (%)  Asian  White  Black or African American  Other  Missing | 170 (45.2)  196 (52.1)  3 (0.8)  1 (0.3)  6 (1.6) | 99 (47.6)  101 (48.6)  3 (1.4)  0  5 (2.4) | 71 (42.3)  95 (56.6)  0  1 (0.6)  1 (0.6) |
| Smoking history, *n* (%)  Current  Former  Never  Missing | 86 (22.9)  93 (24.7)  144 (38.3)  53 (14.1) | 44 (21.2)  37 (17.8)  101 (48.6)  26 (12.5) | 42 (25.0)  56 (33.3)  43 (25.6)  27 (16.1) |
| Disease etiology, *n* (%)  Cirrhosis  HBV  HCV  NASH  ALD  Other | 218 (58.0)  86 (22.9)  30 (8.0)  31 (8.2)  2 (0.5)  9 (2.4) | 79 (38.0)  72 (34.6)  27 (13.0)  30 (14.4)  0  0 | 139 (82.7)  14 (8.3)  3 (1.8)  1 (0.6)  2 (1.2)  9 (5.4) |
| BCLC stage, *n* (%)  0  A  B  C  D | –  –  –  –  – | –  –  –  –  – | 10 (6.0)  67 (39.9)  26 (15.5)  57 (33.9)  8 (4.8) |
| Child-Pugh score, *n* (%)  A  B  C  Missing | 122 (32.5)  41 (10.9)  5 (1.3)  208 (55.3) | 0  0  0  208 (100) | 122 (72.6)  41 (24.4)  5 (3.0)  0 |
| Mean ± SD ALBI score | -2.6 ± 0.7 | -2.9 ± 0.6 | -2.2 ±0.6 |
| ALBI grade, *n* (%)  1  2  3 | 204 (54.3)  147 (39.1)  25 (6.7) | 153 (73.6)  49 (23.6)  6 (2.9) | 51 (30.4)  98 (58.3)  19 (11.3) |
| Ongoing antiviral therapy,  *n* (%)  Yes  No | 105 (27.9)  271 (72.1) | 59 (28.4)  149 (71.6) | 46 (27.4)  122 (72.6) |
| Antibiotics in the last 14 days, *n* (%)  Yes  No | 18 (4.8)  358 (95.2) | 8 (3.9)  200 (96.2) | 10 (6.0)  158 (94.1) |

ALBI, albumin-bilirubin; ALD, alcoholic liver disease; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; SD, standard deviation.

**Table S2** Sensitivity and specificity of the Elecsys PIVKA-II, Lumipulse G PIVKA-II, *µ*TASWako DCP and ARCHITECT PIVKA-II assays for discriminating early-stage HCC cases across different cut-off values, in both mAU/mL and ng/mL

|  |  |  |  |
| --- | --- | --- | --- |
| **Cut-off** | **Assay** | **Sensitivity, % (95% CI)** | **Specificity, % (95% CI)** |
| 20 mAU/mL  (14.2 ng/mL) | Elecsys | 97.4 (90.9–99.7) | 8.7 (5.2–13.3) |
| *µ*TASWako | 85.7 (75.9–92.6) | 48.1 (41.1–55.1) |
| Lumipulse | 89.6 (80.6–95.4) | 22.1 (16.7–28.4) |
| ARCHITECT | 92.2 (83.8–97.1) | 21.6 (16.2–27.9) |
| 30 mAU/mL  (21.3 ng/mL) | Elecsys | 88.3 (79.0–94.5) | 64.9 (58.0–71.4) |
| *µ*TASWako | 76.6 (65.6–85.5) | 80.8 (74.7–85.9) |
| Lumipulse | 80.5 (69.9–88.7) | 66.8 (60.0–73.2) |
| ARCHITECT | 80.5 (69.9–88.7) | 60.6 (53.6–67.3) |
| 40 mAU/mL  (28.4 ng/mL) | Elecsys | 77.9 (67.0–86.6) | 83.7 (77.9–88.4) |
| *µ*TASWako | 68.8 (57.3–78.9) | 89.9 (85.0–93.6) |
| Lumipulse | 72.7 (61.4–82.3) | 84.6 (79.0–89.2) |
| ARCHITECT | 68.8 (57.3–78.9) | 84.6 (79.0–89.2) |
| 60 mAU/mL  (42.6 ng/mL) | Elecsys | 63.6 (51.9–74.3) | 90.9 (86.1–94.4) |
| *µ*TASWako | 58.4 (46.6–69.6) | 95.2 (91.3–97.7) |
| Lumipulse | 58.4 (46.6–69.6) | 93.3 (89.0–96.3) |
| ARCHITECT | 58.4 (46.6–69.6) | 92.3 (87.8–95.5) |
| 80 mAU/mL  (56.8 ng/mL) | Elecsys | 54.5 (42.8–65.9) | 92.8 (88.4–95.9) |
| *µ*TASWako | 53.2 (41.5–64.7) | 96.6 (93.2–98.6) |
| Lumipulse | 55.8 (44.1–67.2) | 95.7 (91.9–98.0) |
| ARCHITECT | 53.2 (41.5–64.7) | 94.7 (90.7–97.3) |
| 100 mAU/mL  (71 ng/mL) | Elecsys | 46.8 (35.3–58.5) | 93.3 (89.0–96.3) |
| *µ*TASWako | 51.9 (40.3–63.5) | 96.6 (93.2–98.6) |
| Lumipulse | 53.2 (41.5–64.7) | 96.6 (93.2–98.6) |
| ARCHITECT | 53.2 (41.5–64.7) | 95.7 (91.9–98.0) |
| 200 mAU/mL  (142 ng/mL) | Elecsys | 40.3 (29.2–52.1) | 97.1 (93.8–98.9) |
| *µ*TASWako | 40.3 (29.2–52.1) | 99.0 (96.6–99.9) |
| Lumipulse | 41.6 (30.4–53.4) | 99.0 (96.6–99.9) |
| ARCHITECT | 41.6 (30.4–53.4) | 98.1 (95.1–99.5) |
| 400 mAU/mL  (284 ng/mL) | Elecsys | 29.9 (20.0–41.4) | 98.6 (95.8–99.7) |
| *µ*TASWako | 26.0 (16.6–37.2) | 100 (98.2–100) |
| Lumipulse | 27.3 (17.7–38.6) | 100 (98.2–100) |
| ARCHITECT | 26.0 (16.6–37.2) | 100 (98.2–100) |
| 1000 mAU/mL  (710 ng/mL) | Elecsys | 11.7 (5.5–21.0) | 100 (98.2–100) |
| *µ*TASWako | 18.2 (10.3–28.6) | 100 (98.2–100) |
| Lumipulse | 18.2 (10.3–28.6) | 100 (98.2–100) |
| ARCHITECT | 18.2 (10.3–28.6) | 100 (98.2–100) |

CI, confidence interval; DCP, des-γ-carboxyprothrombin; HCC, hepatocellular carcinoma; PIVKA-II, prothrombin induced by vitamin K absence-II.

**Table S3** Sensitivity and specificity of the Elecsys PIVKA-II, Lumipulse G PIVKA-II, *µ*TASWako DCP and ARCHITECT PIVKA-II assays for discriminating late-stage HCC cases across different cut-off values, in both mAU/mL and ng/mL

|  |  |  |  |
| --- | --- | --- | --- |
| **Cut-off** | **Assay** | **Sensitivity, % (95% CI)** | **Specificity, % (95% CI)** |
| 20 mAU/mL  (14.2 ng/mL) | Elecsys | 100 (96.0–100) | 8.7 (5.2–13.3) |
| *µ*TASWako | 95.6 (89.1–98.8) | 48.1 (41.1–55.1) |
| Lumipulse | 97.8 (92.3–99.7) | 22.1 (16.7–28.4) |
| ARCHITECT | 97.8 (92.3–99.7) | 21.6 (16.2–27.9) |
| 30 mAU/mL  (21.3 ng/mL) | Elecsys | 96.7 (90.7–99.3) | 64.9 (58.0–71.4) |
| *µ*TASWako | 91.2 (83.4–96.1) | 80.8 (74.7–85.9) |
| Lumipulse | 94.5 (87.6–98.2) | 66.8 (60.0–73.2) |
| ARCHITECT | 94.5 (87.6–98.2) | 60.6 (53.6–67.3) |
| 40 mAU/mL  (28.4 ng/mL) | Elecsys | 94.5 (87.6–98.2) | 83.7 (77.9–88.4) |
| *µ*TASWako | 91.2 (83.4–96.1) | 89.9 (85.0–93.6) |
| Lumipulse | 91.2 (83.4–96.1) | 84.6 (79.0–89.2) |
| ARCHITECT | 91.2 (83.4–96.1) | 84.6 (79.0–89.2) |
| 60 mAU/mL  (42.6 ng/mL) | Elecsys | 90.1 (82.1–95.4) | 90.9 (86.1–94.4) |
| *µ*TASWako | 87.9 (79.4–93.8) | 95.2 (91.3–97.7) |
| Lumipulse | 90.1 (82.1–95.4) | 93.3 (89.0–96.3) |
| ARCHITECT | 87.9 (79.4–93.8) | 92.3 (87.8–95.5) |
| 80 mAU/mL  (56.8 ng/mL) | Elecsys | 87.9 (79.4–93.8) | 92.8 (88.4–95.9) |
| *µ*TASWako | 86.8 (78.1–93.0) | 96.6 (93.2–98.6) |
| Lumipulse | 86.8 (78.1–93.0) | 95.7 (91.9–98.0) |
| ARCHITECT | 85.7 (76.8–92.2) | 94.7 (90.7–97.3) |
| 100 mAU/mL  (71 ng/mL) | Elecsys | 86.8 (78.1–93.0) | 93.3 (89.0–96.3) |
| *µ*TASWako | 84.6 (75.5–91.3) | 96.6 (93.2–98.6) |
| Lumipulse | 86.8 (78.1–93.0) | 96.6 (93.2–98.6) |
| ARCHITECT | 84.6 (75.5–91.3) | 95.7 (91.9–98.0) |
| 200 mAU/mL  (142 ng/mL) | Elecsys | 82.4 (73.0–89.6) | 97.1 (93.8–98.9) |
| *µ*TASWako | 78.0 (68.1–86.0) | 99.0 (96.6–99.9) |
| Lumipulse | 80.2 (70.6–87.8) | 99.0 (96.6–99.9) |
| ARCHITECT | 79.1 (69.3–86.9) | 98.1 (95.1–99.5) |
| 400 mAU/mL  (284 ng/mL) | Elecsys | 70.3 (59.8–79.5) | 98.6 (95.8–99.7) |
| *µ*TASWako | 78.0 (68.1–86.0) | 100 (98.2–100) |
| Lumipulse | 78.0 (68.1–86.0) | 100 (98.2–100) |
| ARCHITECT | 76.9 (66.9–85.1) | 100 (98.2–100) |
| 1000 mAU/mL  (710 ng/mL) | Elecsys | 62.6 (51.9–72.6) | 100 (98.2–100) |
| *µ*TASWako | 64.8 (54.1–74.6) | 100 (98.2–100) |
| Lumipulse | 64.8 (54.1–74.6) | 100 (98.2–100) |
| ARCHITECT | 64.8 (54.1–74.6) | 100 (98.2–100) |

CI, confidence interval; DCP, des-γ-carboxyprothrombin; HCC, hepatocellular carcinoma; PIVKA-II, prothrombin induced by vitamin K absence-II.

**Table S4** Sensitivity and specificity of the Elecsys PIVKA-II, Lumipulse G PIVKA-II, *µ*TASWako DCP and ARCHITECT PIVKA-II assays for discriminating all-stage HCC cases across different cut-off values, in both mAU/mL and ng/mL

|  |  |  |  |
| --- | --- | --- | --- |
| **Cut-off** | **Assay** | **Sensitivity, % (95% CI)** | **Specificity, % (95% CI)** |
| 20 mAU/mL  (14.2 ng/mL) | Elecsys | 98.8 (95.8–99.9) | 8.7 (5.2–13.3) |
| *µ*TASWako | 91.1 (85.7–94.9) | 48.1 (41.1–55.1) |
| Lumipulse | 94.0 (89.3–97.1) | 22.1 (16.7–28.4) |
| ARCHITECT | 95.2 (90.8–97.9) | 21.6 (16.2–27.9) |
| 30 mAU/mL  (21.3 ng/mL) | Elecsys | 92.9 (87.9–96.3) | 64.9 (58.0–71.4) |
| *µ*TASWako | 84.5 (78.2–89.6) | 80.8 (74.7–85.9) |
| Lumipulse | 88.1 (82.2–92.6) | 66.8 (60.0–73.2) |
| ARCHITECT | 88.1 (82.2–92.6) | 60.6 (53.6–67.3) |
| 40 mAU/mL  (28.4 ng/mL) | Elecsys | 86.9 (80.8–91.6) | 83.7 (77.9–88.4) |
| *µ*TASWako | 81.0 (74.2–86.6) | 89.9 (85.0–93.6) |
| Lumipulse | 82.7 (76.2–88.1) | 84.6 (79.0–89.2) |
| ARCHITECT | 81.0 (74.2–86.6) | 84.6 (79.0–89.2) |
| 60 mAU/mL  (42.6 ng/mL) | Elecsys | 78.0 (70.9–84.0) | 90.9 (86.1–94.4) |
| *µ*TASWako | 74.4 (67.1–80.8) | 95.2 (91.3–97.7) |
| Lumipulse | 75.6 (68.4–81.9) | 93.3 (89.0–96.3) |
| ARCHITECT | 74.4 (67.1–80.8) | 92.3 (87.8–95.5) |
| 80 mAU/mL  (56.8 ng/mL) | Elecsys | 72.6 (65.2–79.2) | 92.8 (88.4–95.9) |
| *µ*TASWako | 71.4 (64.0–78.1) | 96.6 (93.2–98.6) |
| Lumipulse | 72.6 (65.2–79.2) | 95.7 (91.9–98.0) |
| ARCHITECT | 70.8 (63.3–77.6) | 94.7 (90.7–97.3) |
| 100 mAU/mL  (71 ng/mL) | Elecsys | 68.5 (60.8–75.4) | 93.3 (89.0–96.3) |
| *µ*TASWako | 69.6 (62.1–76.5) | 96.6 (93.2–98.6) |
| Lumipulse | 71.4 (64.0–78.1) | 96.6 (93.2–98.6) |
| ARCHITECT | 70.2 (62.7–77.0) | 95.7 (91.9–98.0) |
| 200 mAU/mL  (142 ng/mL) | Elecsys | 63.1 (55.3–70.4) | 97.1 (93.8–98.9) |
| *µ*TASWako | 60.7 (52.9–68.1) | 99.0 (96.6–99.9) |
| Lumipulse | 62.5 (54.7–69.8) | 99.0 (96.6–99.9) |
| ARCHITECT | 61.9 (54.1–69.3) | 98.1 (95.1–99.5) |
| 400 mAU/mL  (284 ng/mL) | Elecsys | 51.8 (44.0–59.5) | 98.6 (95.8–99.7) |
| *µ*TASWako | 54.2 (46.3–61.9) | 100 (98.2–100) |
| Lumipulse | 54.8 (46.9–62.4) | 100 (98.2–100) |
| ARCHITECT | 53.6 (45.7–61.3) | 100 (98.2–100) |
| 1000 mAU/mL  (710 ng/mL) | Elecsys | 39.3 (31.9–47.1) | 100 (98.2–100) |
| *µ*TASWako | 43.5 (35.8–51.3) | 100 (98.2–100) |
| Lumipulse | 43.5 (35.8–51.3) | 100 (98.2–100) |
| ARCHITECT | 43.5 (35.8–51.3) | 100 (98.2–100) |

CI, confidence interval; DCP, des-γ-carboxyprothrombin; HCC, hepatocellular carcinoma; PIVKA-II, prothrombin induced by vitamin K absence-II.

**Table S5** Concordance between PIVKA-II and AFP cut-offs by HCC stage

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PIVKA-II cut-off, ng/mL** | **AFP cut-off, ng/mL** | **Concordance between PIVKA-II and AFP cut-offs by HCC stage, %(*n*/*N*)** | | | |
| **Control** | **Early** | **Late** | **All** |
| ≤ 28.4 | ≤ 8.22 | 76 (159/208) | 9 (7/77) | 3 (3/91) | 6 (10/168) |
| > 8.22 | 7 (15/208) | 13 (10/77) | 2 (2/91) | 7 (12/168) |
| ≤ 11.5 | 80 (167/208) | 9 (7/77) | 3 (3/91) | 6 (10/168) |
| > 11.5 | 3 (7/208) | 13 (10/77) | 2 (2/91) | 7 (12/168) |
| > 28.4 | ≤ 8.22 | 14 (29/208) | 35 (27/77) | 24 (22/91) | 29 (49/168) |
| > 8.22 | 2 (5/208) | 43 (33/77) | 70 (64/91) | 58 (97/168) |
| ≤ 11.5 | 14 (30/208) | 39 (30/77) | 26 (24/91) | 32 (54/168) |
| > 11.5 | 2 (4/208) | 39 (30/77) | 68 (62/91) | 55 (92/168) |

AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; PIVKA-II, prothrombin induced by vitamin K absence-II.