

Annex III: *Pig-a* assay performance analysis calculations**Definitions for select metrics:**

	Pig-a P	Pig-a N
Conventional assay P	a	b
Conventional assay N	c	d

% Sensitivity: $a / (a + b) \times 100$

% Positive predictivity: $a / (a + c) \times 100$

% Specificity: $d / (c + d) \times 100$

% Negative predictivity: $d / (b + d) \times 100$

% Concordance: $(a + d) / (a + b + c + d) \times 100$

Performance of *Pig-a* assay responses in relation to different *in vivo* genetic toxicity assay and rodent cancer endpoints: *Pig-a* responses determined using the Working Group (WG) criteria, I and E calls not used.

Pig-a vs BM/blood TGR

	Pig-a P	Pig-a N
BM TGR P	16	0
BM TGR N	1	11

Sensitivity	100% (79%-100%)
Positive predictivity	94% (71%-100%)
Specificity	92% (62%-100%)
Negative predictivity	100% (72%-100%)
Concordance	96% (82%-100%)
Cohen's kappa (95% CI)	0.93 (0.78-1.07)
PABAK	0.93
Discordant test articles	DEN

Pig-a vs TGR

	Pig-a P	Pig-a N
TGR P	26	3
TGR N	0	11

Sensitivity	90% (73%-98%)
Positive predictivity	100% (87%-100%)
Specificity	100% (72%-100%)
Negative predictivity	79% (49%-95%)
Concordance	93% (80%-98%)
Cohen's kappa (95% CI)	0.83 (0.64-1.01)
PABAK	0.85
Discordant test articles	Hydroxyurea, 4-chloro-1,2-diaminobenzine, NNK

Pig-a vs *in vivo* MN

	Pig-a P	Pig-a N
In vivo MN P	29	5
In vivo MN N	2	16

Sensitivity	85% (69%-95%)
Positive predictivity	94% (79%-99%)
Specificity	89% (66%-99%)

Negative predictivity	76% (53%-92%)
Concordance	87% (74%-94%)
Cohen's kappa (95% CI)	0.71 (0.52-0.91)
PABAK	0.73
Discordant test articles	Acetaminophen, aristolochic acids, 4-Chloro-1,2-diaminobenzene, hydroxyurea, vinblastine, CeO ₂ , CEDU

Pig-a vs rodent hematopoietic system cancer

	Pig-a P	Pig-a N
Hematopoietic cancer P	9	1
Hematopoietic cancer N	1	14

Sensitivity	90% (56%-100%)
Positive predictivity	90% (56%-100%)
Specificity	93% (68%-100%)
Negative predictivity	93% (68%-100%)
Concordance	92% (74%-99%)
Cohen's kappa (95% CI)	0.83 (0.61-1.05)
PABAK	0.84
Discordant test articles	Acetaminophen, glycidyl methacrylate

Pig-a vs all rodent cancer

	Pig-a P	Pig-a N
Cancer P	27	7
Cancer N	1	14

Sensitivity	79% (62%-91%)
Positive predictivity	96% (82%-100%)
Specificity	93% (68%-100%)
Negative predictivity	67% (43%-85%)
Concordance	84% (70%-93%)
Cohen's kappa (95% CI)	0.65 (0.44-0.87)
PABAK	0.67
Discordant test articles	Acetaminophen, 2-butoxyethanol, 4-chloro-1,2-diaminobenzene, glycidyl methacrylate, hydroxyurea, clofibrate, methyl carbamate, NNK

Performance of *Pig-a* assay responses in relation to different *in vivo* genetic toxicity assay and rodent cancer endpoints: *Pig-a* responses determined using Extended Criteria, I and E calls not used.

Pig-a vs BM/blood TGR

	Pig-a P	Pig-a N
BM TGR P	15	0
BM TGR N	0	11

Sensitivity	100% (78%-100%)
Positive predictivity	100% (78%-100%)
Specificity	100% (72%-100%)
Negative predictivity	100% (72%-100%)
Concordance	100% (87%-100%)
Cohen's kappa (95% CI)	1.00 (1.00-1.00)
PABAK	1.00
Discordant test articles	

Pig-a vs TGR

	Pig-a P	Pig-a N
TGR P	22	4
TGR N	0	11

Sensitivity	84% (65%-96%)
Positive predictivity	100% (85%-100%)
Specificity	100% (72%-100%)
Negative predictivity	79% (45%-92%)
Concordance	89% (75%-97%)
Cohen's kappa (95% CI)	0.77 (0.56-0.98)
PABAK	0.78
Discordant test articles	Hydroxyurea, 4-chloro-1,2-diaminobenzine, NNK, 1,2-dimethylhydrazine

Pig-a vs *in vivo* MN

	Pig-a P	Pig-a N
In vivo MN P	25	9
In vivo MN N	2	17

Sensitivity	74% (56%-87%)
Positive predictivity	93% (76%-99%)
Specificity	89% (67%-99%)
Negative predictivity	65% (44%-83%)
Concordance	79% (66%-89%)
Cohen's kappa (95% CI)	0.583 (0.37-0.79)

PABAK	0.58
Discordant test articles	Acetaminophen, aristolochic acids, 4-Chloro-1,2-diaminobenzene, AZT, caffeic acid, 1,2-dimethylhydrazine, 5-fluorouracil, hydroxyurea, vinblastine, CeO ₂ , CEDU

Pig-a vs rodent hematopoietic system cancer

	Pig-a P	Pig-a N
Hematopoietic cancer P	9	1
Hematopoietic cancer N	1	15

Sensitivity	90% (56%-100%)
Positive predictivity	90% (56%-100%)
Specificity	94% (70%-100%)
Negative predictivity	94% (70%-100%)
Concordance	92% (75%-99%)
Cohen's kappa (95% CI)	0.84 (0.621-1.05)
PABAK	0.85
Discordant test articles	Acetaminophen, glycidyl methacrylate

Pig-a vs all rodent cancer

	Pig-a P	Pig-a N
Cancer P	22	12
Cancer N	1	15

Sensitivity	65% (46%-80%)
Positive predictivity	96% (78%-100%)
Specificity	94% (70%-100%)
Negative predictivity	56% (35%-75%)
Concordance	74% (60%-85%)
Cohen's kappa (95% CI)	0.50 (0.28-0.71)
PABAK	0.48
Discordant test articles	Acetaminophen, 2-butoxyethanol, caffeic acid, AZT, 4-chloro-1,2-diaminobenzene, clofibrate, 1,2-dimethylhydrazine, 5-fluorouracil, glycidyl methacrylate, hydroxyurea, melamine, methyl carbamate, NNK

Performance of *Pig-a* assay responses in relation to different *in vivo* genetic toxicity assay and rodent cancer endpoints: *Pig-a* responses using only short-term treatment protocols and determined using Extended Criteria, I and E calls not used.

Pig-a vs BM/blood TGR

	Pig-a P	Pig-a N
BM TGR P	13	0
BM TGR N	0	14

Sensitivity	100% (75%-100%)
Positive predictivity	100% (75%-100%)
Specificity	100% (77%-100%)
Negative predictivity	100% (77%-100%)
Concordance	100% (87%-100%)
Cohen's kappa (95% CI)	1.00 (1.00-1.00)
PABAK	1.00
Discordant test articles	

Pig-a vs TGR

	Pig-a P	Pig-a N
TGR P	20	4
TGR N	0	10

Sensitivity	83% (63%-95%)
Positive predictivity	100% (83%-100%)
Specificity	100% (69%-100%)
Negative predictivity	71% (42%-92%)
Concordance	88% (73%-97%)
Cohen's kappa (95% CI)	0.75 (0.52-0.97)
PABAK	0.77
Discordant test articles	Hydroxyurea, DEN, aflatoxin B1, 1,2-dimethylhydrazine

Pig-a vs *in vivo* MN

	Pig-a P	Pig-a N
In vivo MN P	22	8
In vivo MN N	2	17

Sensitivity	73% (54%-88%)
Positive predictivity	92% (73%-99%)
Specificity	89% (67%-99%)
Negative predictivity	68% (47%-85%)
Concordance	80% (66%-90%)
Cohen's kappa (95% CI)	0.59 (0.38-0.81)
PABAK	0.59

Discordant test articles	Acetaminophen, aristolochic acids, hydroxyurea, aflatoxin B1, AZT, caffeic acid, DEN, dimethylhydrazine, 5-fluorouracil, CEDU
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Pig-a vs rodent hematopoietic system cancer

	Pig-a P	Pig-a N
Hematopoietic cancer P	9	1
Hematopoietic cancer N	0	14

Sensitivity	90% (56%-100%)
Positive predictivity	100% (66%-100%)
Specificity	100% (77%-100%)
Negative predictivity	93% (68%-100%)
Concordance	96% (79%-100%)
Cohen's kappa (95% CI)	0.91 (0.75-1.08)
PABAK	0.92
Discordant test articles	Acetaminophen

Pig-a vs all rodent cancer

	Pig-a P	Pig-a N
Cancer P	21	11
Cancer N	0	14

Sensitivity	66% (47%-81%)
Positive predictivity	100% (84%-100%)
Specificity	100% (77%-100%)
Negative predictivity	56% (35%-76%)
Concordance	76% (61%-87%)
Cohen's kappa (95% CI)	0.54 (0.33-0.75)
PABAK	0.52
Discordant test articles	Acetaminophen, aflatoxin B1, AZT, 2-butoxyethanol, caffeic acid, 5-fluorouracil, hydroxyurea, melamine, methyl carbamate, DEN, 1,2-dimethylhydrazine