GENETIC TOXICOLOGY SCREENING: A COVANCE PERSPECTIVE

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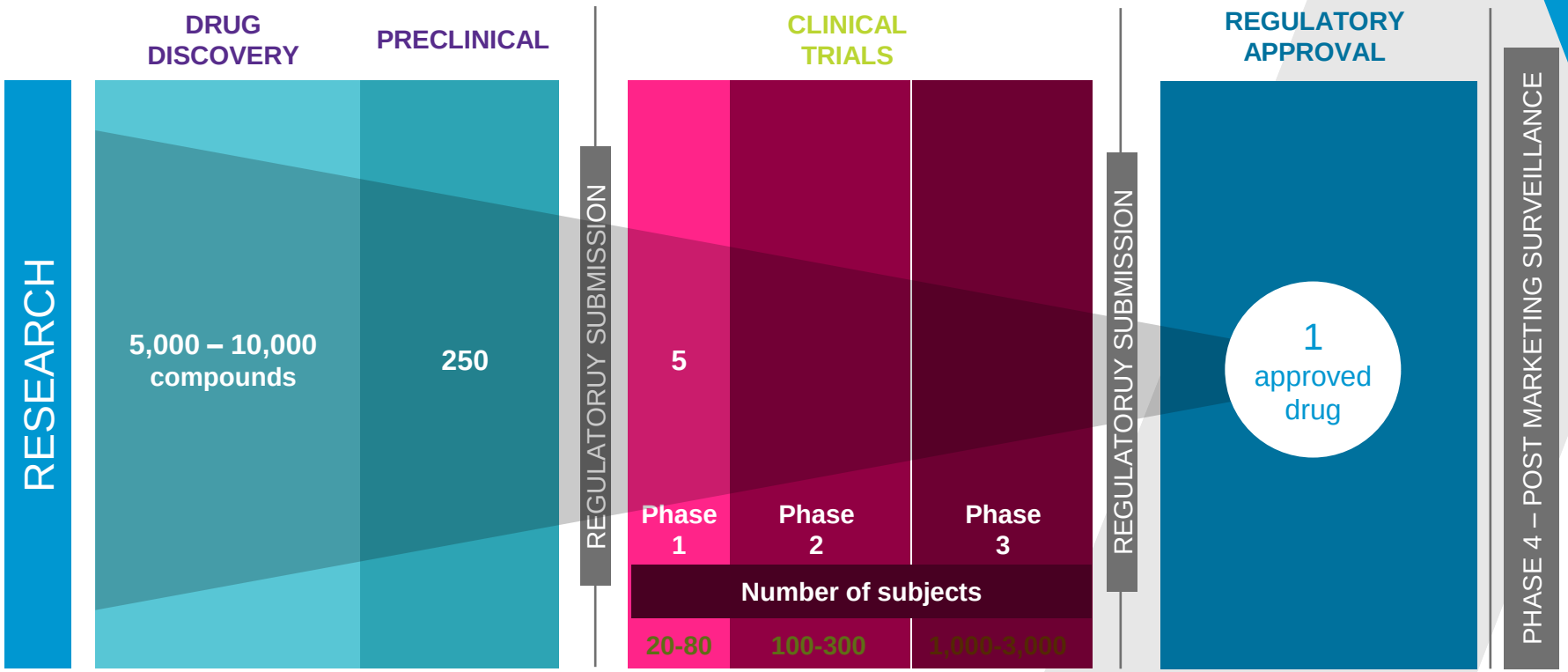
Navigating drug discovery and development



Genetic Toxicology Screening: Rationale for Early Evaluation

- Screening allows early identification of potential genotoxicity
  - Focus time and resource on most promising compounds
  - Efficient planning for follow-up testing
  - Reduces ’surprises’ in later development
- Advantages of screening assays
  - Reduced test article requirement (miniaturised designs)
  - Quicker turn-around time
  - Lower cost compared to regulatory assays
  - Predictive of regulatory tests
- One approach is to mimic the regulatory study as closely as possible for best predictivity
  - Design dependent on test article availability

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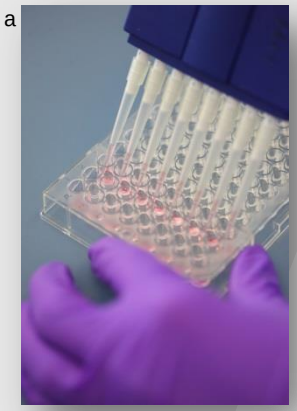

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CRO Perspective

- Historically, only GLP and Pre-GLP studies were outsourced to CRO
  - Early screening with mg of compound performed in-house
- Industry has moved towards outsourcing earlier in development
- This has created challenges and opportunities
  - Miniaturisation of regulatory assays with minimal compound (to predict GLP assay outcome)
  - Turn around times (TATs) for multiple clients
  - Client specific requirements (try to mimic in-house design/strategy)
- Streamlined process to provide high quality data with reduced TATs

Genetic Toxicology Screening Assays

- Most regulatory genetic tox assays can be scaled down for screening (to conserve test article, time, etc.)
- Screening should ideally cover gene mutation and chromosome damage
  - Bacterial Mutation Assay (Ames)
  - *In vitro* micronucleus
- Different designs based on availability of test article

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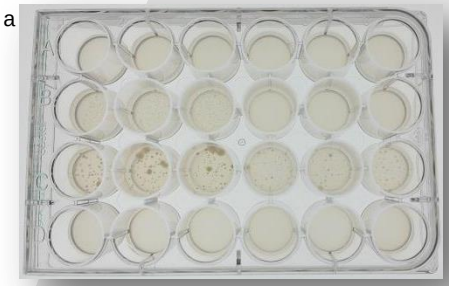

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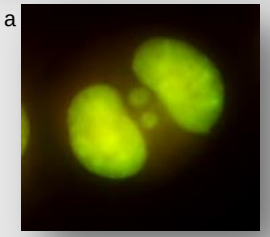

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Ames Screen: Abbreviated and Multi-Well Modifications

			
Format	Standard	6-Well	24-Well
Area	~500 mm^2	~90 mm^2	~20 mm^2
Maximum concentration	5000 µg/plate	1000 µg/well*	250 µg/well*
Compound per strain	40 to 50 mg per strain	15 mg per strain	3 mg per strain
*Equivalent to 5000 µg/plate			

- Strains TA98 and TA100 minimum screen

Ames: Standard v Multi-Well Screens
Historical Vehicle Control Data

- Ranges comparable between standard and multi-well
  - increased variability with multi-well assay (low revertant numbers)
- Recommendations/Considerations with low spontaneous revertant strains
  - Use strain TA97a in place of TA1537
  - Use strain WP2 uvrA pKM101 for *E.coli* strain
  - Use historical data to help assess biological relevance of small increases (e.g. strains TA98 and TA1535)

	Revertant Numbers			
	Mean Revertants per Plate		99% Reference Range	
Strain	Standard Ames	24-Well Ames	Standard Ames	24-Well Ames
TA98	24	2	10 to 46	0 to 7
	34	3	15 to 56	0 to 11
TA100	113	7	76 to 170	1 to 15
	123	7	81 to 157	0 to 15
TA1535	22	2	7 to 45	0 to 7
	18	1	9 to 34	0 to 4
TA1537	10	1	2 to 25	0 to 3
	18	1	7 to 30	0 to 4
TA97a	113	8	74 to 155	1 to 19
	184	10	105 to 249	3 to 19
TA102	270	25	185 to 362	15 to 40
	269	29	179 to 393	11 to 45
WP2 *uvrA*	171	15	121 to 241	7 to 25
pKM101	219	20	148 to 303	11 to 32

Ames: Standard v Multi-Well Screens Positive/Negative Comparison

Validation Compounds	Expected Outcome	Actual Outcome			Comparison	
		24-well plate	90-mm plate	6-well plate	24-well vs 90-mm	6-well vs 90-mm
2-Aminoanthracene	+	+	+	+	✓	✓
Anthracene	–	–	–	–	✓	✓
ICR-191	+	+	+	+	✓	✓
Benzo[a]pyrene	+	+	+	+	✓	✓
Sodium azide	+	+	+	+	✓	✓
4-Methoxycarbonyl-phenylboronic acid	Unknown	–	–	–	✓	✓
1-H-Pyrazole-4-boronic acid	Unknown	+	+	+	✓	✓
4-Nitroquinoline	+	+	+	+	✓	✓
2-Nitrofluorine	+	+	+	+	✓	✓
7,12-Dimethylbenzanthracene	+	+	+	+	✓	✓
L-Methionine	–	–	–	–	✓	✓

The 24- and 6-well format Ames assay correctly predicts the overall outcome in the standard 90-mm plate Ames assay

- Multi-well formats correctly predict overall outcome from standard Ames assay

Ames: Standard v Multi-Well Screens
24-Well v 90 mm Plates

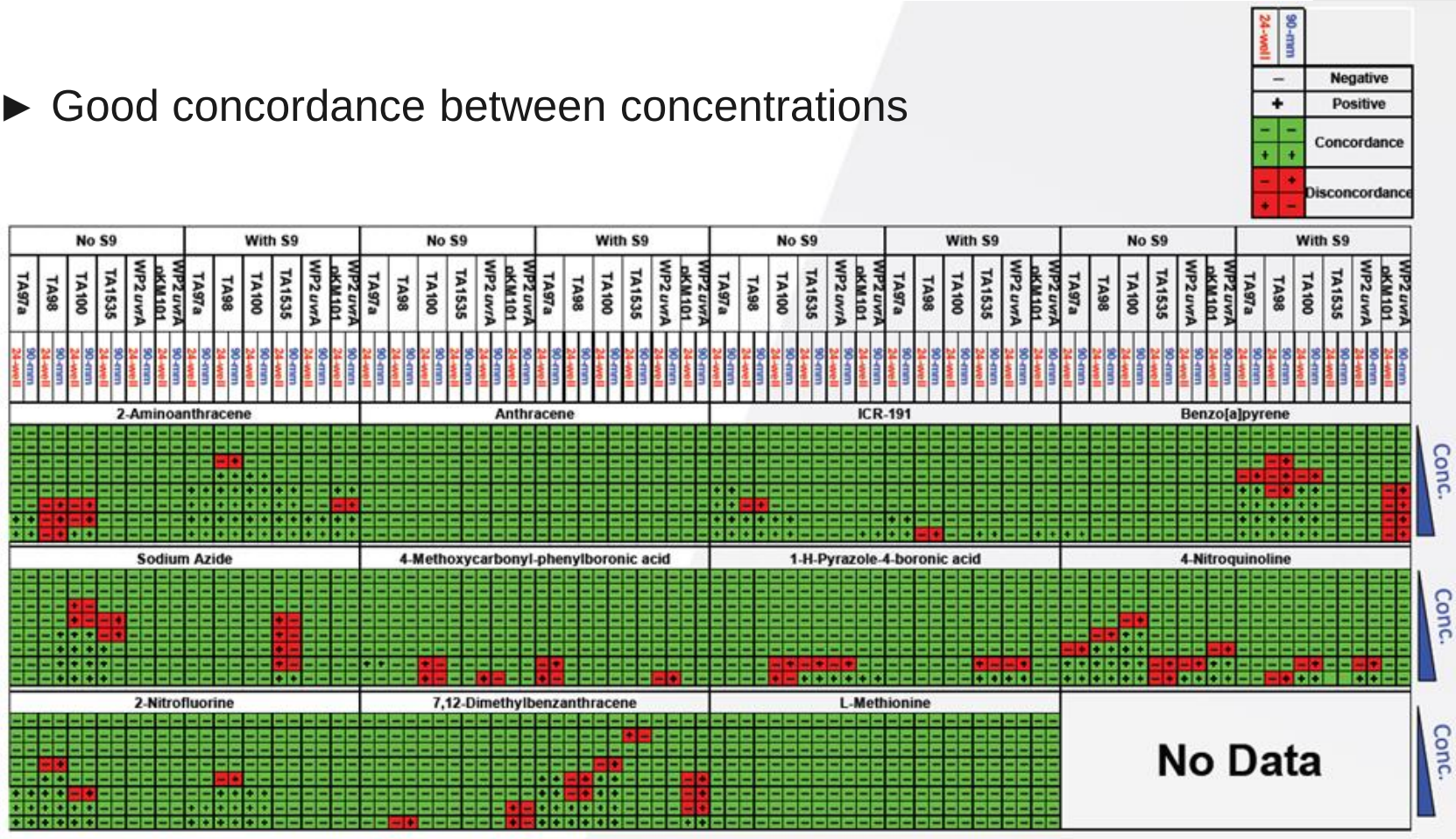
	TA97a		TA98		TA100		TA1535		WP2*uvrA*		WP2uvrA pKM101	
	No S9	+ S9	No S9	+ S9	No S9	+ S9	No S9	+ S9	No S9	+ S9	No S9	+ S9
	2-Aminoanthracene											
24-well plate assay	+	+	–	+	–	+	–	+	–	+	–	+
90-mm plate assay	+	+	+	+	+	+	–	+	–	+	–	+
24-well vs 90-mm plates	✓	✓	×	✓	×	✓	✓	✓	✓	✓	✓	✓
	Anthracene											
24-well plate assay	–	–	–	–	–	–	–	–	–	–	–	–
90-mm plate assay	–	–	–	–	–	–	–	–	–	–	–	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	ICR-191											
24-well plate assay	+	+	+	–	+	–	–	–	–	–	–	–
90-mm plate assay	+	+	+	–	+	–	–	–	–	–	+	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓
	Benzo[a]pyrene											
24-well plate assay	–	+	–	+	–	+	–	–	–	–	–	–
90-mm plate assay	–	+	–	+	–	+	–	–	–	–	–	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Sodium azide											
24-well plate assay	–	–	–	–	+	–	+	–	–	–	–	–
90-mm plate assay	–	–	–	–	+	–	+	+	–	–	–	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓
	4-Methoxycarbonyl-phenylboronic acid											
24-well plate assay	–	–	–	–	+	–	–	–	–	–	–	–
90-mm plate assay	–	–	–	–	–	–	–	–	–	–	–	–
24-well vs 90-mm plates	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓

	TA97a		TA98		TA100		TA1535		WP2*uvrA*		WP2uvrA pKM101	
	No S9	+ S9	No S9	+ S9	No S9	+ S9	No S9	+ S9	No S9	+ S9	No S9	+ S9
	1-H-Pyrazole-4-boronic acid											
24-well plate assay	–	–	–	–	–	–	–	–	–	–	–	–
90-mm plate assay	–	–	–	–	–	–	+	+	+	+	–	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	✓	×	×	×	×	✓	✓
	4-Nitroquinoline											
24-well plate assay	+	–	+	–	+	–	–	–	–	–	+	–
90-mm plate assay	+	–	+	–	+	+	+	–	+	+	+	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	×	×	✓	×	×	✓	✓
	2-Nitrofluorine											
24-well plate assay	+	+	+	+	+	+	–	–	–	–	–	–
90-mm plate assay	+	+	+	+	+	+	–	–	–	–	–	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	7,12-Dimethylbenzanthracene											
24-well plate assay	–	+	–	+	–	+	–	–	–	–	+	–
90-mm plate assay	+	+	–	+	–	+	–	–	–	–	–	+
24-well vs 90-mm plates	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	×
	L-Methionine											
24-well plate assay	–	–	–	–	–	–	–	–	–	–	–	–
90-mm plate assay	–	–	–	–	–	–	–	–	–	–	–	–
24-well vs 90-mm plates	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

- Good concordance between strains (multi-well v standard Ames)

Ames: Standard v Multi-Well Screens Concentration by Concentration

- Good concordance between concentrations



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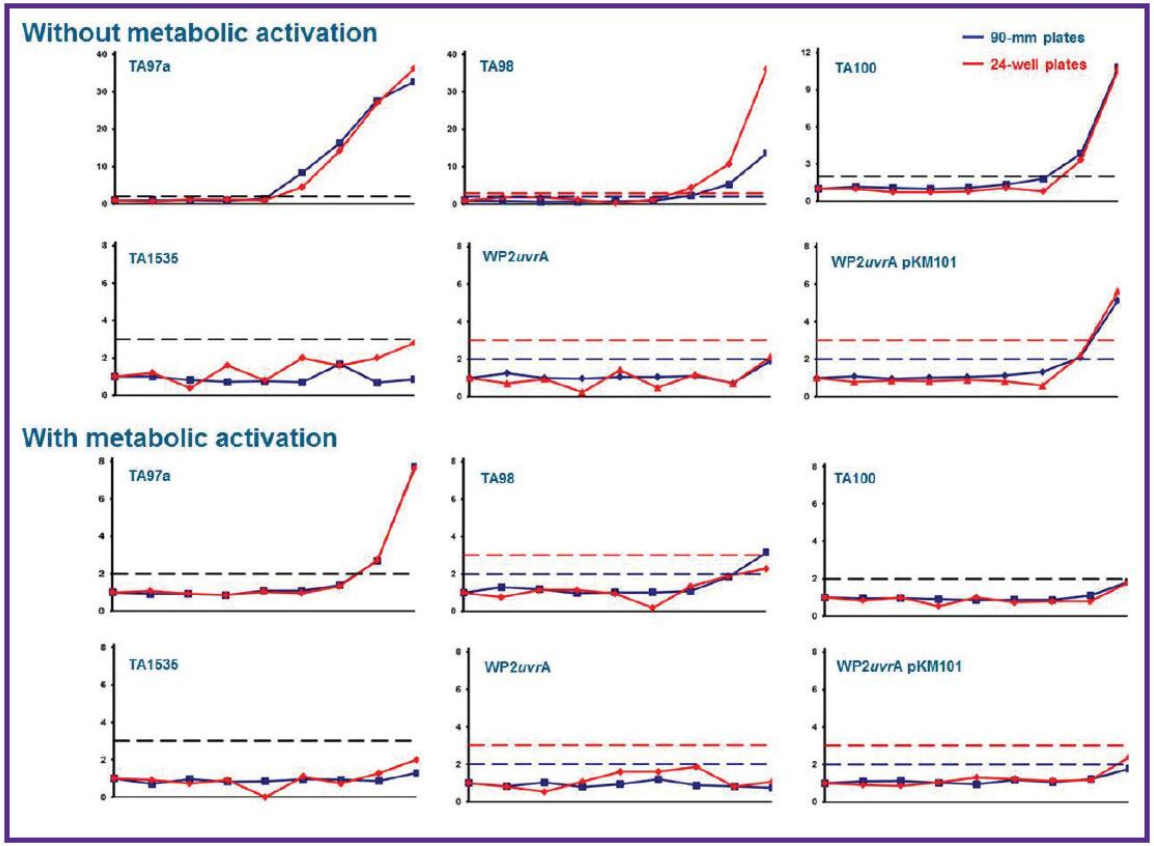
Ames Screens: Standard v Multi-Well Concentration by Concentration

90-mm vs 24-Well Plates, ICR-191



- Corresponding concentrations show good concordance (multi-well v standard Ames)

Ames Screens: Pros / Cons

- Multi-Well modifications generally show good concordance to the standard plate assay
  - Outcome (positive / negative)
  - Concentration
- Current OECD initiative: Can mini-Ames formats be used as surrogate for Ames assay in certain circumstances?
- Can use the same strains
- Significantly less test article usage
- Target follow-up testing in specific strains to investigate equivocal results (increase replicate wells or in full plate)

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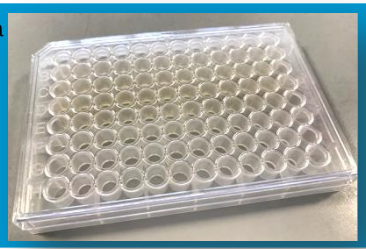
- Considerations with low spontaneous revertant strains
  - Use appropriate strains
  - Use of historical ranges aids interpretation

In Vitro MN Screens: Abbreviated and 96-Well Formats

	a 	a 
Format	Tube	96-Well
Volume	5 / 10 mL	150 µL
Maximum concentration	1 mM / 500 µg/mL Cytotoxicity limit for MN analysis	
Compound	50 to 200 mg	5 mg
Cells	Human PBLs or TK6, Mouse L5178	TK6

- Extended treatment, -S-9 & Short treatment +S-9 minimum
- Same cytotoxicity measures
- Other volumes and cell lines can be used
- Reduced BN cells (500 to 1000 per concentration)
- Semi-automated MN analysis using Metafer

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In Vitro MN: Standard v 96-Well Historical vehicle controls

	MNBN Cell Frequency (%)			
	3+27 Hour +S9 (with Cyto B)		27+27 Hour -S9 (with Cyto B)	
	Standard TK6	Micro TK6	Standard TK6	Micro TK6
Mean	0.60	0.69	0.68	0.82
Observed Range	0.10 to 1.30	0 to 1.40	0.30 to 2.10	0 to 2.00
95% Reference Range	0.12 to 1.20	0.02 to 1.29	0.36 to 1.78	0.20 to 1.81

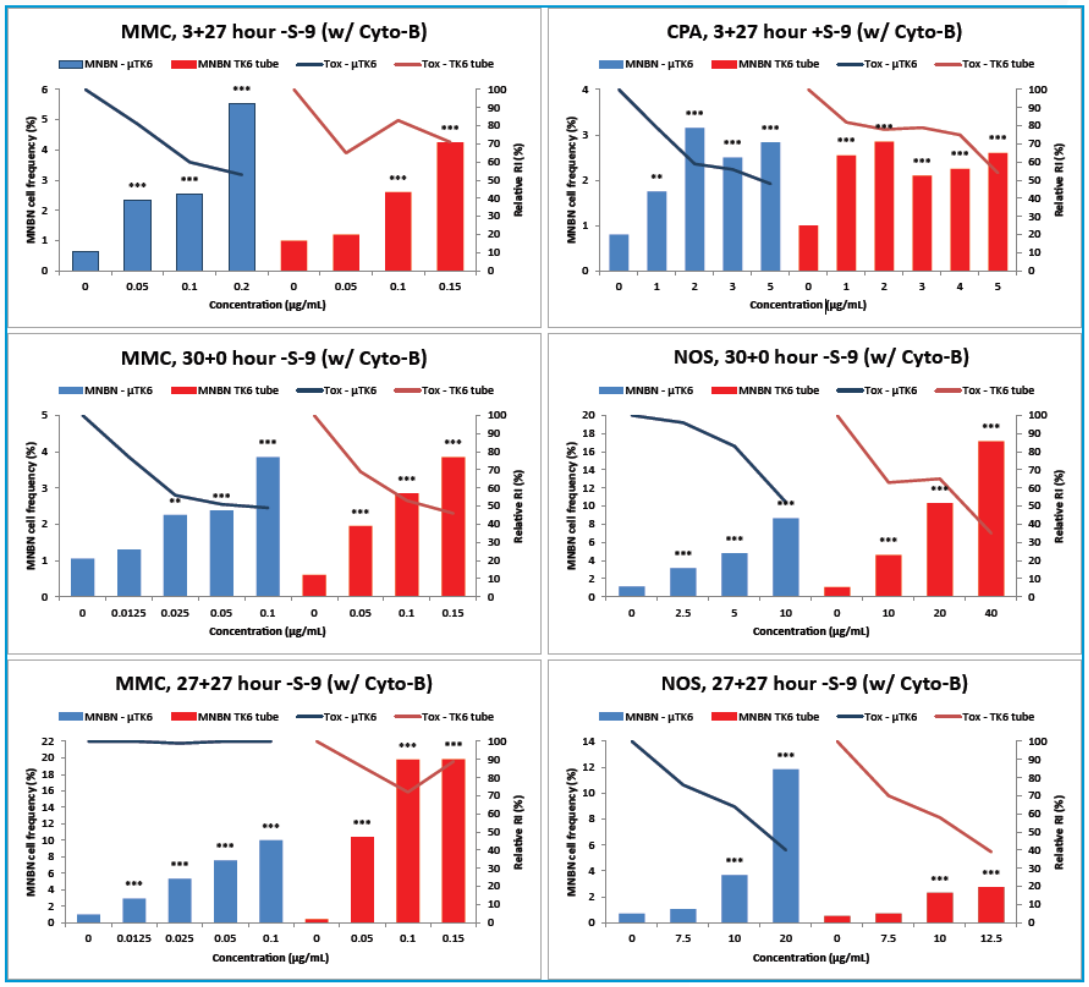
- Historical ranges comparable (96-well v standard tube format)

In Vitro MN: Standard v 96-Well Positive / Negative Comparison

Test Chemical	Expected Outcome	Actual Outcome				Comparison
		96-Well micro TK6		Culture Tube TK6		
		w/ Cyto-B	w/o Cyto-B	w/ Cyto-B	w/o Cyto-B	
Saline	-	–	–	–	–	✓
Cyclophoshamide (CPA)	+	+	+	+	+	✓
Benzo(a)pyrene	+	+	+	+	+	✓
Mitomycin C (MMC)	+	+	+	+	+	✓
Vinblastine (VIN)	+	+	+	+	+	✓
Noscapine (NOS)	+	+	+	+	+	✓
Colchicine	+	not tested	+	not tested	+	✓
Cytosine Arabinoside	+	not tested	+	not tested	+	✓
Methyl Methanesulfonate	+	not tested	+	not tested	+	✓

- Good concordance between standard tube and 96-Well formats

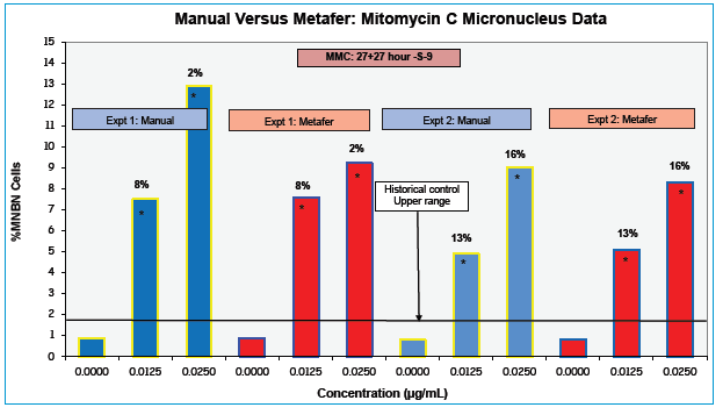
In Vitro MN: Standard v 96-Well Scoring

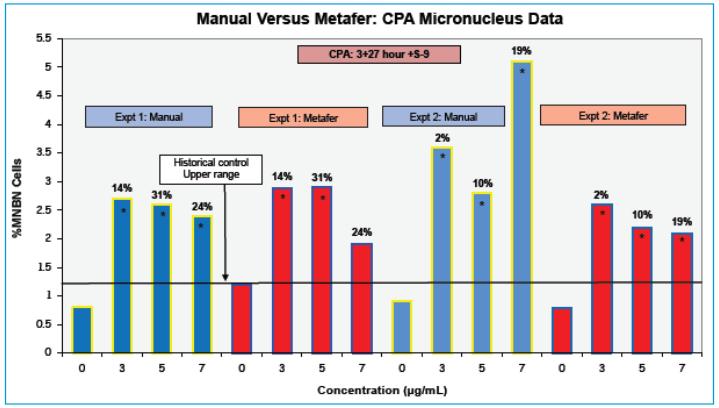


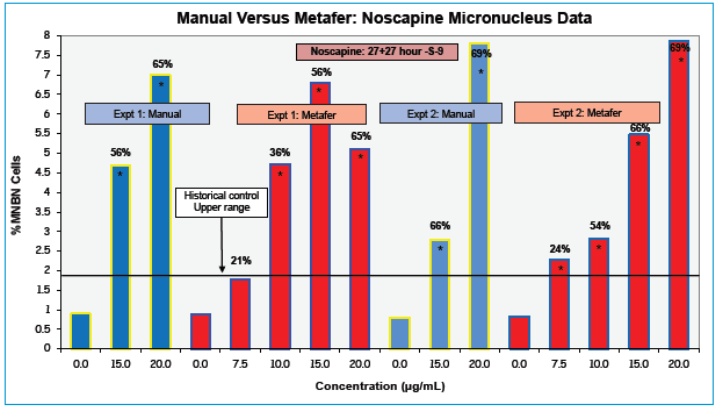
- Good concordance between standard tube and 96-Well formats

In Vitro MN Screen: Manual v Metafer Analysis

Manual versus Semi-Automated Metafer Analysis







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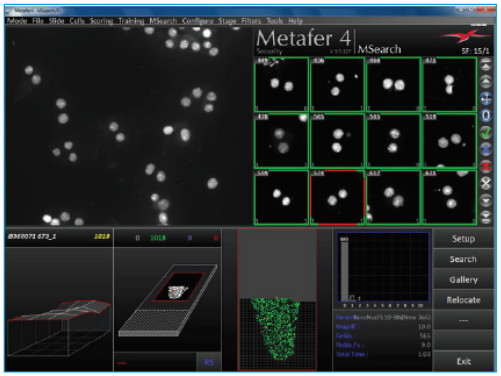

Metafer MSearch gallery showing MN analysis.

In Vitro MN Screen: Pros / Cons

- 96-well format generally show good concordance with standard tube based assay
  - Outcome (positive / negative)
  - Good concentration concordance
- Significantly less test article used
- Use of (semi-)automation reduces turn around times

- Fewer cells scored
- May not evaluate all three treatment conditions

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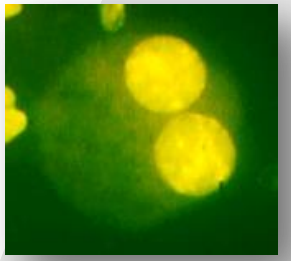

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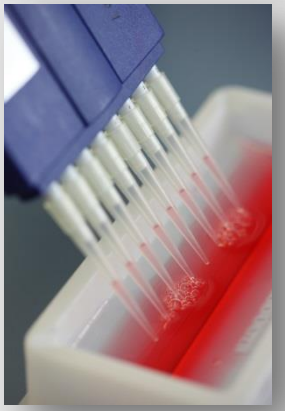

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Conclusion

- Early Screening allows earlier identification of genotoxic compounds
  - Saves valuable time and resource
- Miniaturised screens require significantly less test article
- Miniaturised screens have good concordance with corresponding regulatory assays

WHAT ARE THE NEXT STEPS FOR SCREENING…

- Future developments/techniques may improve turnaround time and provide additional relevant information with limited compound
- Automated micronucleus analysis
  - Automated image based platforms
  - Flow cytometry
  - Imaging flow cytometry (e.g. ImageStream)
- Additional/multiple endpoints – e.g. MultiFlow™
  - Aneugen, Clastogen or Non-genotoxic
  - Mode of Action

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