Bacterial urinary mutagenicity test for assessment of cigarette smoke

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Contents

1 Introduction

Urinary biomarker

- **A biomarker** is an indicator of a biological state, or the past or present existence of a particular type of organism. (Wikipedia)

- **Urinary biomarkers** are,
  non-invasive & correlated with toxic and metabolic response
  recommended to assess the exposure of smokers to smoke constituents

Smoke Component	Urinary Biomarker of Exposure
Nicotine	* Total nicotine metabolites [nicotine, nicotine-glucuronide cotinine, cotinine-glucuronide THC(trans-3'-hydroxycotinine), THC-glucuronide]
Acrolein	3-Hydroxypropyl mercapturic acid (3-HPMA)
1,3-Butadiene	Monohydroxybutenyl-mercapturic acids(MHBMA)
Mutagen	Urine Mutagenicity

1 Introduction

Urinary mutagenicity test

- **The urinary mutagenicity** is a biomarker for assessing the systemic production of mutagenic compounds by the body

- **The urinary mutagenicity test** is most useful when:

  1) the specific chemical is unknown
  2) analytical techniques are not available
  3) exposure is to undefined complex mixtures

1 Introduction

Urinary mutagenicity studies related to cigarette smoking

- Many studies have demonstrated the dependency of urinary mutagenicity on cigarette smoking (IARC, 2004).

- An increase in urinary mutagenicity was significantly correlated with each external and internal indicators of exposure to tobacco smoke (Sofia P. *et al*., 2003)

- The urine of tobacco-heating cigarette smokers is less mutagenic than urine of tobacco-burning cigarette smokers (Smith CJ *et al*., 1996)

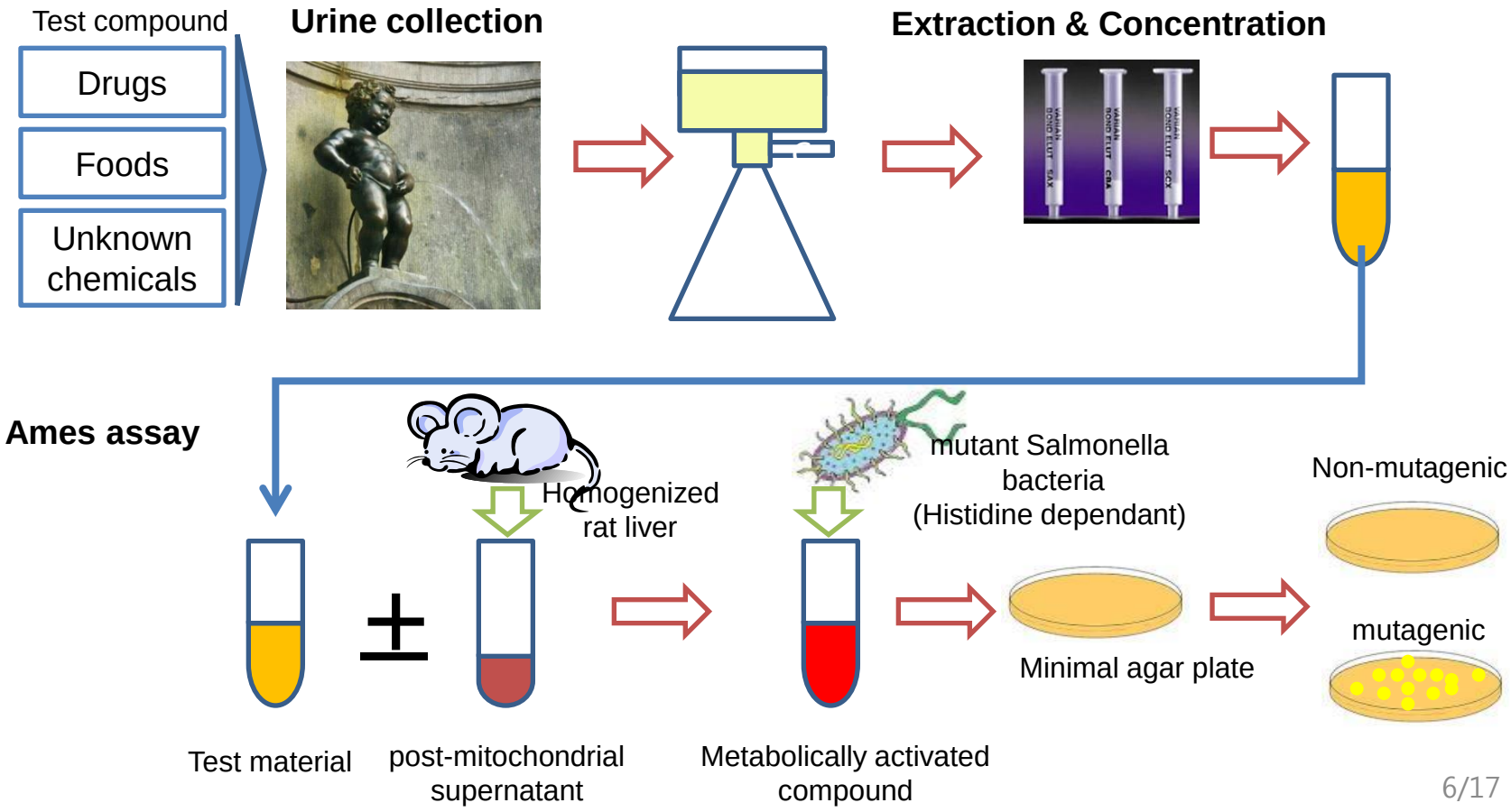
- Methodologies for the detection of urinary mutagens from donor smokers were optimised. (Ballantyne M. *et al*., 2008)

Urinary mutagenicity is recognized as a useful biomarker of cigarette smoke exposure

1 Introduction

Urinary mutagenicity test

- based on Ames assay
- need to be optimized & validated to detect low urinary levels of mutagens



1 Introduction

Objectives

- Optimization & Establishment

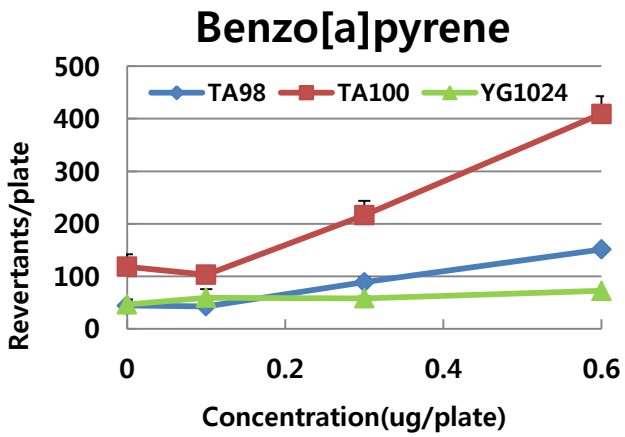
1) In-house urinary mutagenicity assay for enhancing the mutagenic response
2) Urine Extraction and Concentration Techniques for minimizing possible interference

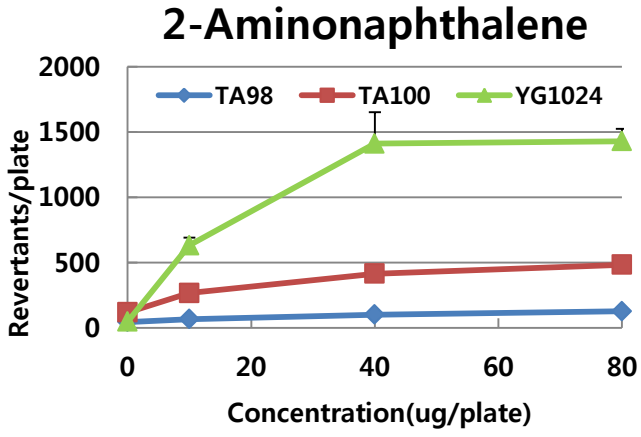
- Validation & sensitivity analysis

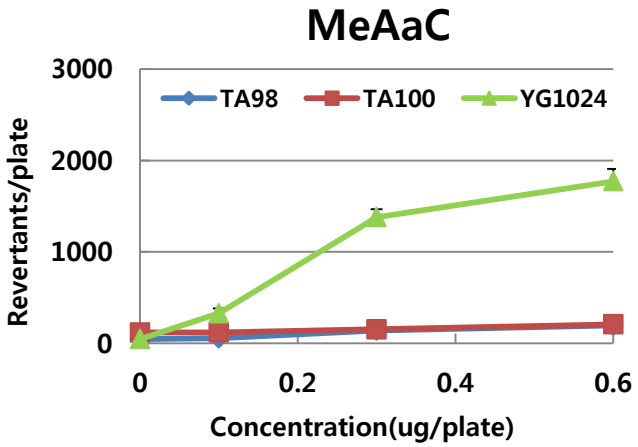
1) Smokers vs. Non-smoker
2) Heavy smoker vs. Light smoker
3) Analysis of relationship with total nicotine metabolites

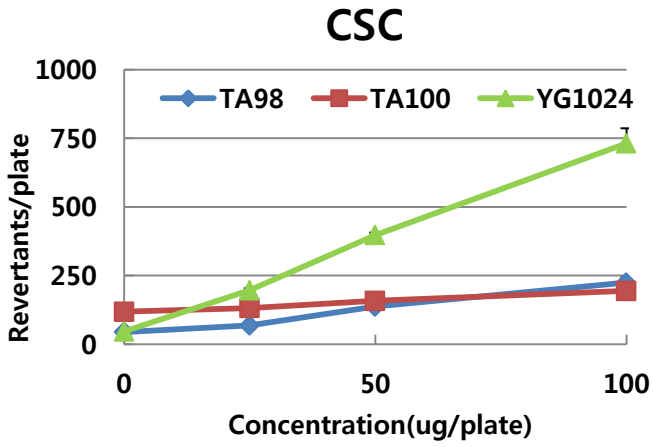
2 Optimization of urinary mutagenicity test

Selection of test strain









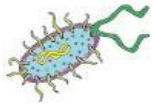
* MeAaC (2-Amino-3-methyl-9H-pyrido[2,3-b]indole)

- The mutagenicity was most effective at YG1024 strain.

2 Optimization of urinary mutagenicity test

Optimization of Ames assay

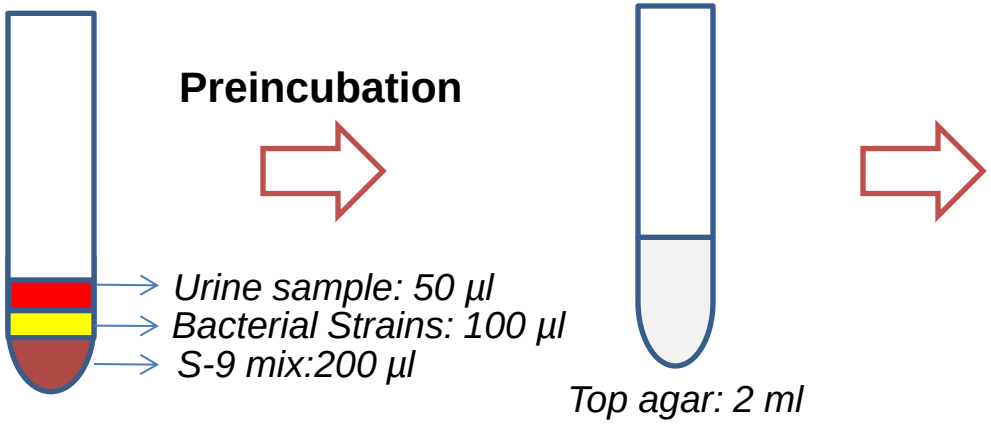
Strains: YG 1024



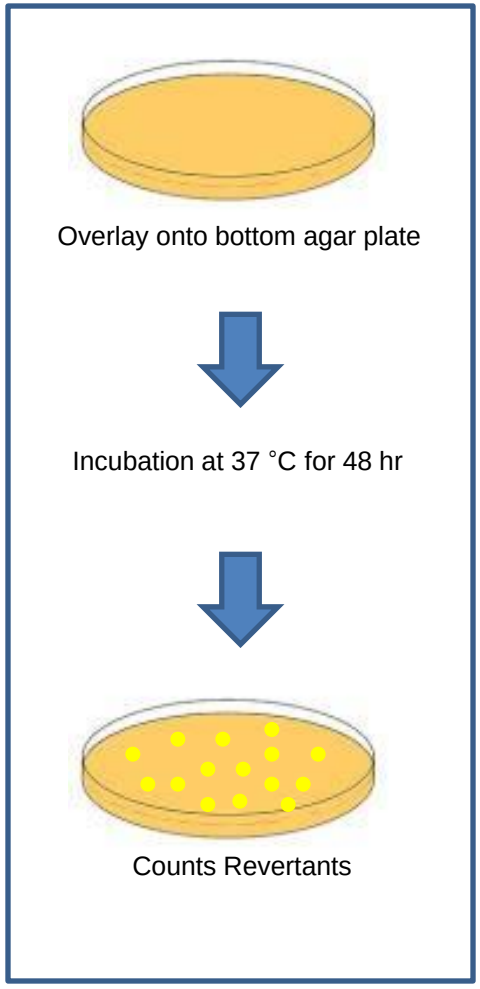
- *overproducing o-acetyltransferase*
- *more sensitive in detecting mutagenicity caused by cigarette smoke*

Microsuspension Method

- *More sensitive than plate incorporation method*



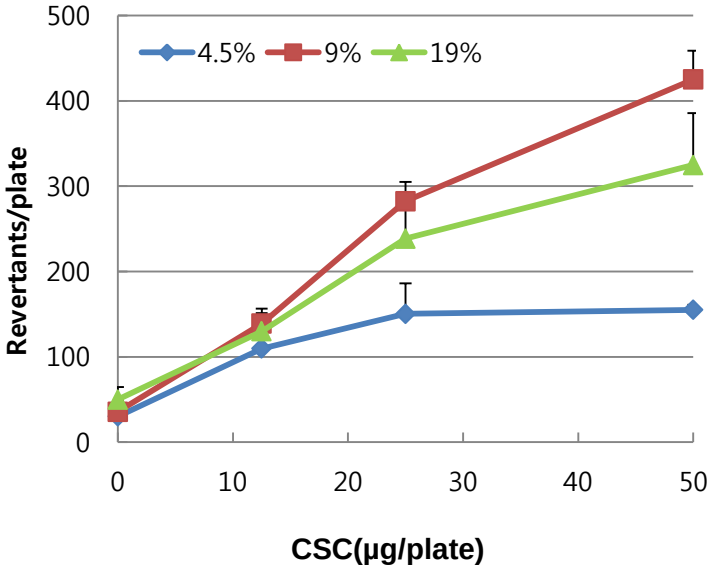
S9 mix Conc.: 4.5%, 9%, 19%
Preincubation time: 20, 40, 90 minutes

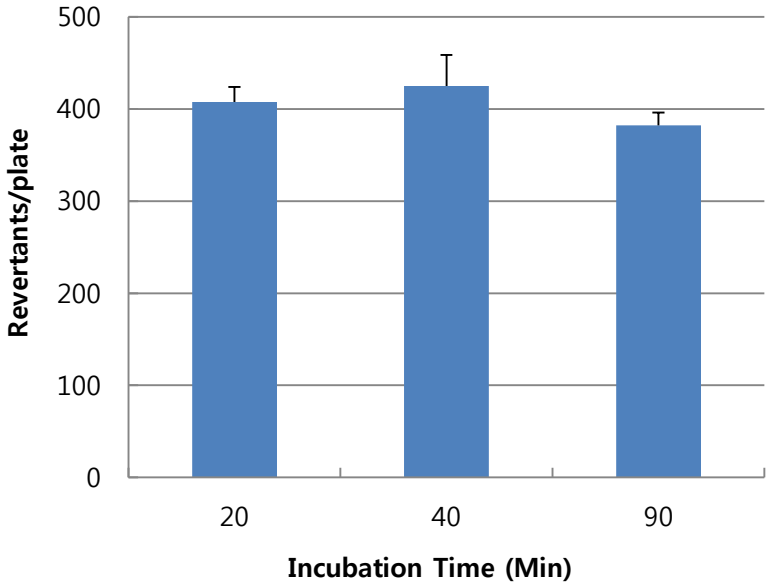


2 Optimization of urinary mutagenicity test

Optimization of Ames assay

Influence of S9 concentration and incubation time on the sensitivity of Microsuspension Method





- *The mutagenicity was most effective at a concentration of 9% (v/v) liver S9.*
- *A longer incubation time than 40 min resulted in decrease in the number of revertant colonies*

2 Optimization of urinary mutagenicity test

Urine Extraction and Concentration

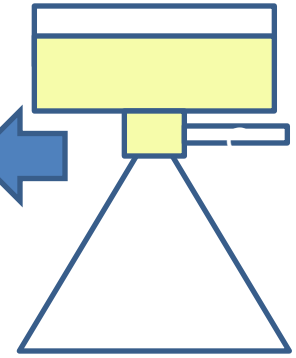
24 hr
urine
2~4 L

Centrifuge
At 2500 x g
For 20 min

Adjust pH to 7.0 with 10N NaOH

24 hr
urine
supernatant

Filtration: glass microfiber filter (1µm)



Aliquot
200 ml

SPE vacuum manifolds
Varian Mega bond C18 cartridge (2g sorbent)

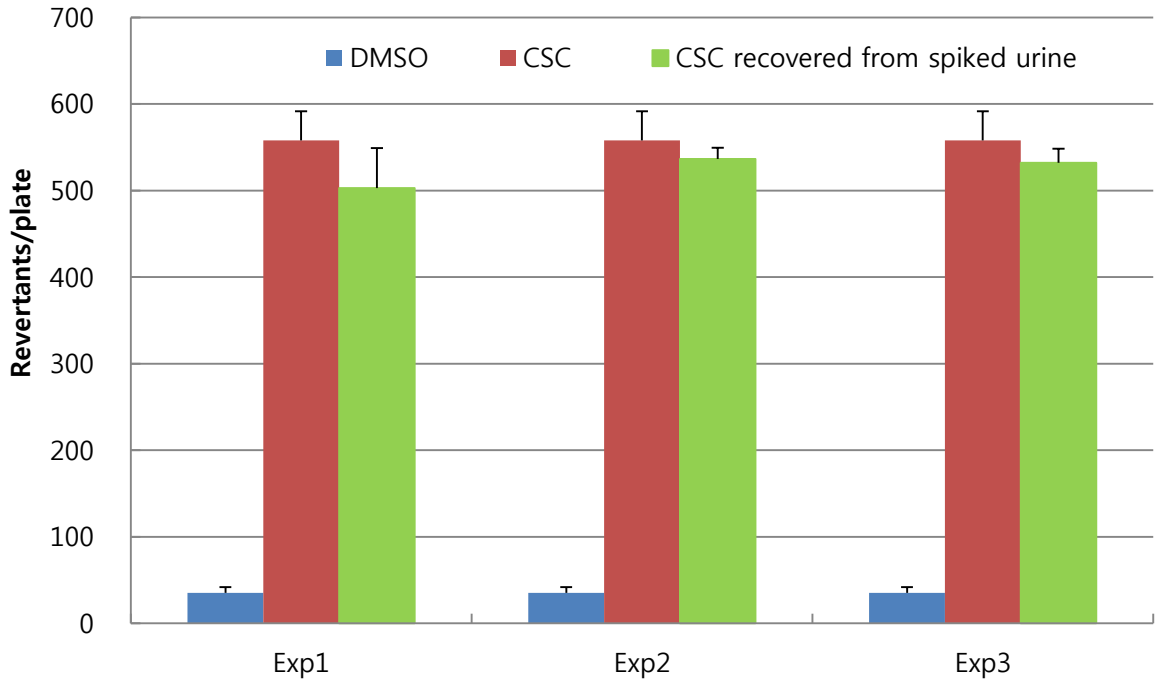
Conditioning: MeOH 10ml→Water 10 ml
Sample application: 200 ml
Washing: Water 10 ml (5ml x 2)
Elution: 10 ml (5ml x 2) of MeOH:Acetonitrile (1:1)
Evaporation: 47°C (using nitrogen gas)
Resuspension: 1ml DMSO

1 ml
(X 200)

2 Optimization of urinary mutagenicity test

Urine Extraction and Concentration

Spike and recovery experiments for validating the urine extraction & concentration method



CSC.: Cigarette Smoke Condensate
Spiked Conc.: 4 mg

- The recovery rate of spiked CSC was nearly 90% in urine matrix.

3 Validation study

Pilot study design

Subjects

- Number of participants : smokers(n=7, S1~S7), non-smoker(n=1, NS)
- Gender : Male
- Mean age : 43
- Number of cigarette smoked : 5-30 per day

  Heavy smoker group (n=4), Light smoker group (n=3)

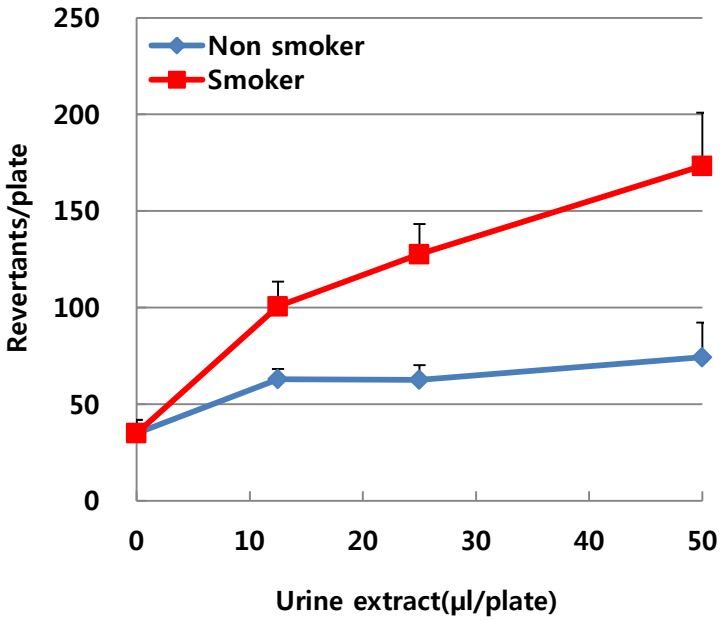
Urine collection

- 24hr Urine samples from participants were obtained and stored at -70℃.

3 Validation study

Results

Comparison of urine mutagenicity between smokers and non-smoker



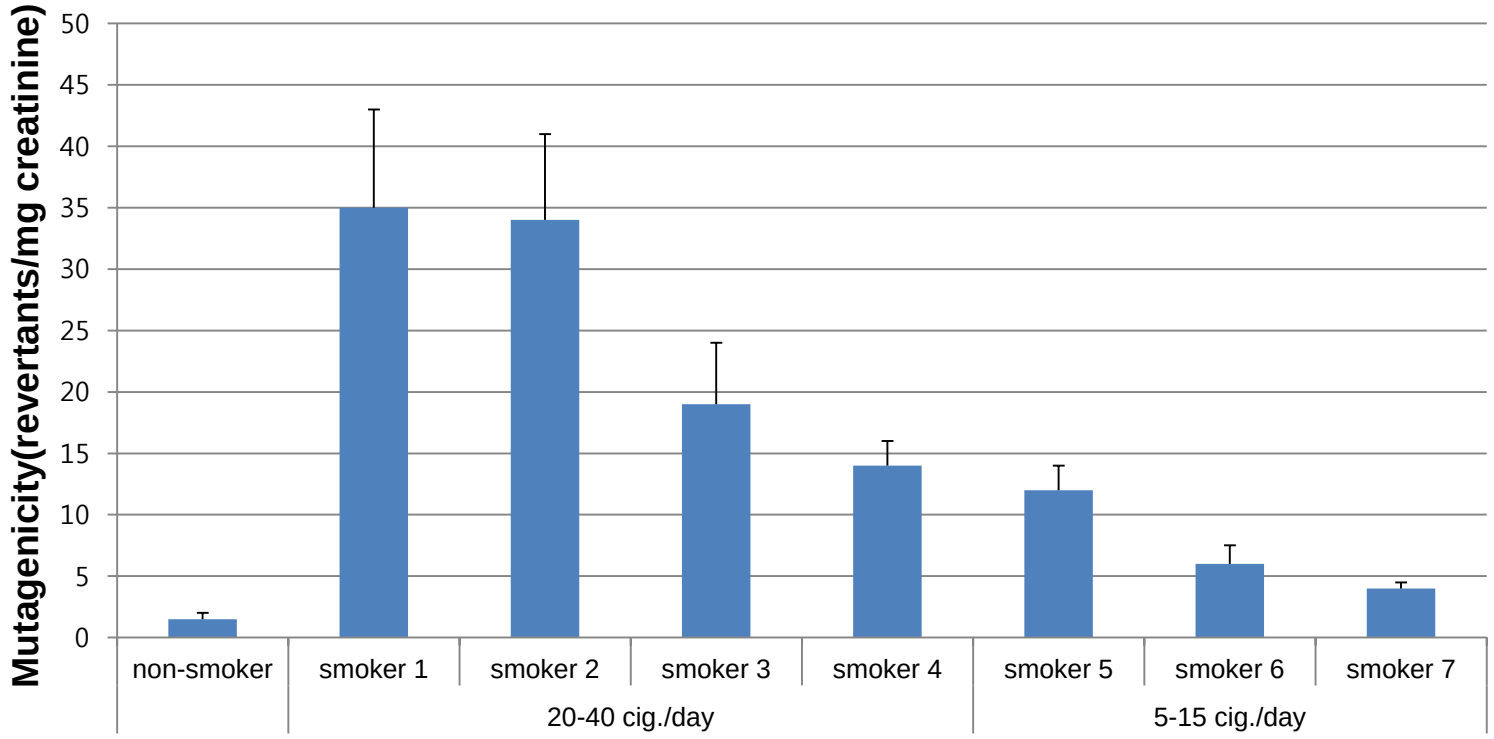
Urine	Batch	Mutagenicity (number of rev./mg creatinine)
Non-smoker	Batch 1	5.30±0.98
	Batch 2	6.77±0.68
	Batch 3	5.02±1.21
Smoker	Batch 1	31.56±0.62
	Batch 2	42.63±0.59
	Batch 3	31.57±0.68

- *Creatinine correction can reduce the variability of body size and urinary output*
- *After the creatinine adjustment, the mutagenicity of smoker's urine was, on average, 6-fold higher than that of non-smoker's urine.*

3 Validation study

Results

- Urine mutagenicity of individual smokers

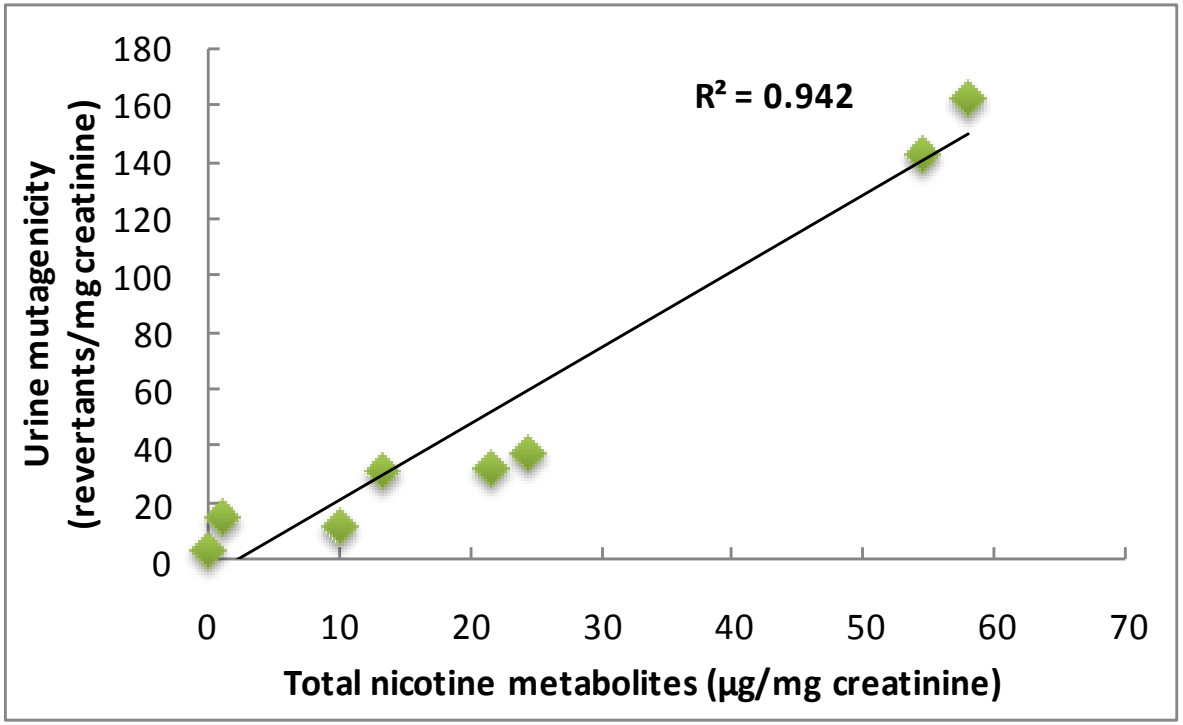


- Individual creatinine-adjusted urinary mutagenicity was highly associated with cigarette smoke exposure levels

3 Validation study

Results

- Relationship with total nicotine metabolites



Total nicotine metabolites

1.Nicotine
2.Nicotine-glucuronide
3.Cotinine
4.Cotinine-glucuronide
5.THC(trans-3'-hydroxycotinine)
6.THC-glucuronide

- Urinary mutagenicity may be an effective biomarker of cigarette smoke.

4 Conclusion

We optimized the several parameters & experimental condition to improve the sensitivity of urine mutagenicity.

- The most appropriate strain
- the concentration of metabolic activation system & preincubation time
- the urine extraction and concentration process & techniques

Our method was validated by comparing smoker with non-smoker and heavy smoker with light smoker

- There is significant difference in urine mutagenicity between the smokers and non-smoker.
- Our method can differentiate heavy smoker from light smoker.
- As a smoke exposure biomarker, urinary mutagenicity was correlated with total nicotine metabolites.

Urinary mutagenicity test could be used as part of the evaluation of cigarettes that is designed to have the potential to reduce smoking-related diseases

THANK YOU FOR YOUR ATTENTION