

Supplementary Material

Supplementary Methods

Analytical Validation of miRNA Assays

The analytical performance of the miRNA assays used for gastric cancer biomarker identification was evaluated. We first evaluated the analytical specificity of these assays by conducting a cross-reactivity test of miRNA assays against 9 highly homologous let-7 family members (**Figure S2A**), a design routinely used to evaluate assay specificity. The let-7 family assays were able to discriminate homologous sequences with even single nucleotide differences, e.g. let-7a assay showed 100% detection of let-7a target and only 0.8% cross-reactivity against let-7c target. Secondly, we evaluated the reproducibility of the miRNA assays by measuring 200 circulating miRNAs in 30 control and cancer serum specimen in two independent labs (**Figure S2B**). After normalization of technical and biological variations using the multi-layered controls illustrated in **Figure S1**, these assays demonstrated encouraging concordance of 0.95-0.98 in all 30 clinical samples. Lastly, we evaluated the analytical sensitivity of the miRNA assays (**Figure S2C**). Constrained by the small size, miRNA assay performances can be highly variable across different miRNA targets, especially miRNAs with higher AT content. We selected 8 commonly studied miRNAs with low to high AT content (36.4% - 63.6%) and compared the analytical sensitivity and dynamic range of the MiRXES miRNA assays against the well known Taqman probe based assays. The miRNA assays used for gastric cancer biomarker discovery demonstrated consistent amplification and detection of all 8 miRNA targets across at least 7 logs of dynamic range where the probe-based assays showed less consistent performance, especially against miRNA targets with higher AT content. Overall, these validation studies demonstrated good analytical performance of the assays and the workflow, and warrant their use for biomarker discovery.

Laboratory Procedures for miRNA Expression Quantification in Discovery and Verification Phases

Spike-In Controls for RT-qPCR Workflow

To monitor and normalize technical variations in RNA isolation efficiency, a set of 3 proprietary synthetic miRNAs were spiked into the sample lysis buffer (Qiazol) at high, medium and low concentrations. To monitor and normalize technical variations in subsequent RT and qPCR reactions, a second set of 3 proprietary synthetic miRNAs were then spiked into each isolated sample RNA at high, medium and low concentrations. A 6-log serial dilution of synthetic templates (10^7 to 10^2 copies) for each miRNA, non-template control (nuclease-free water spiked with MS2) and reference human serum RNA were concurrently reverse-transcribed and quantified by qPCR with each isolated serum RNA sample. These control measures facilitated monitoring and normalization of technical variations in pipetting and assay efficiency in RT, cDNA amplification, and qPCR.

Determination and Normalization of Absolute miRNA Expressions

Upon completion of RT-qPCR, Ct values were determined using the ViiA 7 RUO software (Thermo Fisher Scientific Inc, USA) with automatic baseline setting and a threshold of 0.5. Absolute expression of each miRNA in patient serum was determined through intra-polation of synthetic miRNA standard curves and corrected for RT-qPCR efficiency variation using spike-in RNAs. The miRNA expression of each sample was further normalized using 6 endogenous reference miRNAs independently identified using the geNorm and NormFinder reference gene algorithms [1, 2]. The miRNA expression profiles normalized using the 6 reference miRNAs were similar to that normalized by global mean expression of all miRNA quantified. Absolute expression of miRNAs were log2 transformed for subsequent statistical analysis and optimization of multivariate biomarker panel.

Multivariate Analysis For Constructing Multi-miR Panels

A linear support vector machine (SVM) was used to construct the multi-variant biomarker panels and the associated algorithm that classified cancer and control groups with highest AUC. Multiple iterations of four-

fold cross-validation (matched by sex, cancer subtype and disease stage) were conducted to evaluate the performance of these panels. All calculations were performed using Matlab® software (MathWorks, USA).

Laboratory Procedures for 12-miR Multi-Target Assay in Validation Phase

The assay involved 3 steps: (1) RNA isolation from serum samples; (2) cDNA synthesis; and (3) Detection of miRNAs by quantitative PCR (qPCR). Extraction of RNA was performed by combining phenol/guanidine-based lysis of serum sample and silica-membrane-based purification of total RNA. During cDNA synthesis, 12 miRNA targets from each specimen were converted into cDNAs using 12 corresponding miRNA-specific stem-loop-based reverse transcription primers in a single reaction on a Veriti Dx thermocycler (Thermo Fisher Scientific Inc, USA). At the qPCR step, each miRNA target was amplified by a sequence-specific forward PCR primer and a hemi-nested sequence specific reverse PCR primer and detected using SYBR Green I dye in single-plex reactions on a Quantstudio Dx (384-well) real-time qPCR instrument (Thermo Fisher Scientific Inc, USA). Ct values of the 12 biomarker and reference miRNAs were exported using the QuantStudio Dx Software v1.0.1 (Thermo Fisher Scientific Inc, USA) and converted into a single numerical score using a validated, prespecified logistic-regression algorithm through the GASTROSmart Software (MIRXES Pte Ltd, Singapore). In each assay run, 13 patient specimens were processed concurrently with 2 quantitative reference specimen and 1 negative control specimen, which served as quality control and inter-run normalizers.

Cost-effectiveness analysis

We examined the cost-effectiveness of implementing the miRNA biomarker panel as a screen before endoscopy in a proposed national screening program in Singapore. Our study focusses on the cohort of Singaporean Chinese males, age 50-75 years, who are at an intermediate risk of gastric cancer, and compare the proposed mass screening program with the current pattern of gastric cancer diagnosis without screening. Chinese population carry ~90% of gastric cancer disease burden in Singapore with males at a 30% higher risk of gastric cancer than females [3, 4]. With the cancer incidence rising sharply after the age of 50 years⁴, this subgroup with intermediate gastric cancer risk has a 4 times higher annual incidence rate than the general population. We estimate the quality-adjusted life years (QALY), costs per person, incremental cost-effectiveness ratio (ICER) and also the benefits of early cancer diagnosis and reduced mortality achieved by implementing the mass screening program.

Detailed research methodology of cost-effectiveness analysis is as follows:

- **Target Population:** The analysis is performed on the cohort of Singaporean Chinese males aged 50-75 years.
- **Interventions Compared**
The two interventions compared are:
 1. Current practice of no screening.
 2. Mass screening program using miRNA -test, followed with test-positive patients undergoing a confirmatory upper-endoscopy and biopsy and test-negative subjects to be followed up 3-yearly.
- **Methodology**
Markov decision model was built in Microsoft Excel 2010 to compare the two interventions in the target population by analyzing in a closed cohort setting (**Figure S7**). Model was populated using local and published data with the cohort size estimated from 2016 population census [5]. With a healthcare system perspective, a 25 years' time horizon was analyzed with subjects exiting the model at the age of 75 years. Subjects were expected to be in one of the five health conditions – healthy (cancer-free), TNM Stage 1, TNM Stage 2, TNM Stage 3 and untreatable terminal stage (Stage 4). Early or advanced stage patients (stage 1, 2, and 3) received curative treatment with a stage specific cancer recurrence possibility after a mean duration of 2 years [6, 7], while terminal

cancer patients (stage 4) received only palliative care with a conditional life expectancy of 1 year [6]. As prognosis of the cancer recurrence is poor, patients diagnosed with recurrence were assumed untreatable (equivalent to stage 4) and were given palliative care. Only gastric cancer related mortality was compared as the background mortality due to natural or other causes was expected to be similar in both the scenarios.

The current practice of no screening evaluates the costs, health impacts and mortality as per the current diagnosis rate of gastric cancer in this specific population cohort. We used the published age-specific annual incidence rates of gastric cancer and stage of diagnosis among Chinese Males in Singapore. In the current practice of no-screening we did not account for the cost of false positive endoscopies and the diagnostic expenses of only the true cancer cases was considered, which was a conservative assumption favoring no-screening, similar to the assumption in earlier studies [8, 9]. The proposed mass screening program on the other hand was expected to screen the compliant cohort, identify the cancer cases early due to regular screening and computes the cost, health impacts and mortality accordingly. The subjects tested negative in the screening program will include both healthy cases and missed cancer cases. The missed cancer patients were expected to experience the consequences of treatment delays – disease progression, impact on cost and quality-of-life and an increased mortality, as the cancer would progress in them undiagnosed and untreated and the healthy cases are expected to remain healthy with a possibility of developing gastric cancer in future. Cancers missed in the mass screening program were considered to progress to advanced stages and are expected to be diagnosed at stage 4 due to presentation of symptoms in clinics where they are investigated by endoscopy and biopsy. A 1-year progression time was estimated between the consecutive cancer stages, i.e. a missed stage 1 cancer is expected to progress to stage 2 and then to stage 3 and stage 4 with a one year gap each between the successive stages. Stage 4 patients which were missed in diagnosis were expected to be diagnosed after a mean time of 2 months due to worsening of symptoms.

The compliance rate for mass screening was assumed to be 45% as per the reported compliance in national gastric cancer screening programs in Korea [10] with the non-compliant group expected to behave similar to the current strategy of no-screening. As the miRNA test is simpler to administer and potentially cheaper than the currently used screening methods of UGIS, X-ray or endoscopy, it is hoped to improve the population compliance rate. Thus the performance of the mass screening program across a range of compliance rates (45% - 100%) was also evaluated. All costs quoted in US dollars have been calculated based on the exchange rate of \$1.38 Singapore dollars to 1 USD as per exchange rates in July 2017. All costs and health benefits were discounted at an annual rate of 3%.

- **Scenario and sensitivity analysis:** The cost advantages and non-invasive nature of miRNA testing may increase patient compliance with screening relative to current technologies. Scenarios that capture a range of improved compliance rates (45%–100%) were modeled to evaluate the possible impact on early diagnosis (**Figure S5**). An extensive sensitivity analyses was conducted by varying the values of key parameters—endoscopy cost, miRNA test costs, miRNA test specificity/sensitivity by cancer stages (stages 1, 2, 3, 4), QoL values by cancer stages (stage 1, 2, 3, 4), cancer recurrence rates by the stage at diagnosis and average annual incidence of gastric cancer—to evaluate model robustness at a Willingness-to-Pay threshold of 50,000 USD/ QALY (**Figure S8, 9, 10**).
- **Treatment Protocols for Cancer Treatment and Related Costs:** Stage-specific treatment protocols and average medical expenditures for gastric cancer were obtained from the National University Hospital and expert opinions of clinicians based on current

practices in Singapore (Supplementary Table 11). Patients diagnosed with gastric cancer undergo staging investigation, which includes Computerized Tomography (CT), Chest X-Ray (CX-R), Endoscopic Ultrasound (EUR) and a specialist consultation (including the cost of nurse counseling and an estimated round-trip transport). Curative treatment administered to stage 1, 2, and 3 cancer patients includes surgery (total/ partial gastrectomy) and hospital stay of 12 days. Stage 3 patients undergo an additional chemo-radiotherapy (5 follow-ups) and radiotherapy sessions (5 sessions/ week for 5 weeks). Palliative care for stage 4 patients includes bypass surgery (30%), endoscopic stenting (6%), palliative chemotherapy-5 sessions (16%) and conservative treatment (2x specialist visits) (48%) with an appropriate hospital stay (12 days in surgery cases and 2.5 days for cases with no surgery). Patients are also expected to adhere to follow up visits (average 2.2 visits/ 5 years) and repeat CT and CXR (average 1.5 per year for 5 years after the diagnosis). The miRNA panel test cost in Singapore has been assumed to be USD 30 with an additional 10% for handling and administrative purposes. However, the cost of organizing mass screening has not been included. Costs and QALYs were presented on a present-value basis, with an annual discount rate of 3%. All the diagnosed cases are expected to undergo a biopsy examination. Total costs have been evaluated inclusive of Goods and Services Tax (GST) and without considering any government subsidy.

- **Quality of life values:**

Stage-specific EQ-5D quality-of-life (QoL) index measures were obtained from a previous local study [11] performed on Chinese gastric cancer patients in National University Hospital, Singapore (Table S6). A diagnosed patient is expected to be immediately started on treatment, and experience the diagnosed stage-specific QoL for 1 year with a 6-month additional decrease in QoL due to the initial surgery referred as disutility. After one year of treatment, the patient is expected to enjoy a QoL equivalent to an asymptomatic patient (similar to stage 1 cancer) for the remaining time until faced with any recurrence, which would subsequently drop the QoL to stage 4 equivalent (Table S6).

- **Test Characteristics:**

Test characteristics for diagnostic endoscopy with biopsy for the suspected cases (sensitivity: 93%, specificity: 100%) has been resourced from a study evaluating diagnostic accuracy through a retrospective study among gastric cancer patients [12]. Biopsy is believed to be perfect with 100% sensitivity and specificity. The miRNA stage –specific sensitivity and specificity as estimated from the Singapore Discovery Cohort have been considered as the base-case value.

- **Estimation of population prevalence of undiagnosed gastric cancer:**

As the study aim to identify the benefits of early diagnosis of gastric cancer, it is essential to calculate the population prevalence of undiagnosed gastric cancer cases in the target group. The current annual age-specific incidence rate is 57 cancers per 100,000 in this population cohort [4] with a stage specific distribution of stage 1: 2:3: 4:: 18%:12%:27%:43% . Based on the assumption of 1 year time for progression of cancer from one stage to another, undiagnosed cancer prevalence in the population cohort (stage 1 and higher) was evaluated individually before every mass screening follow-up. Also, the stage 1 and 2 cancers which currently develop and are diagnosed in between the follow-up years are expected to continue to be diagnosed in both the strategies.

Supplementary Figures

Figure S1. Multi-layered control measures for absolute quantification of miRNA expression

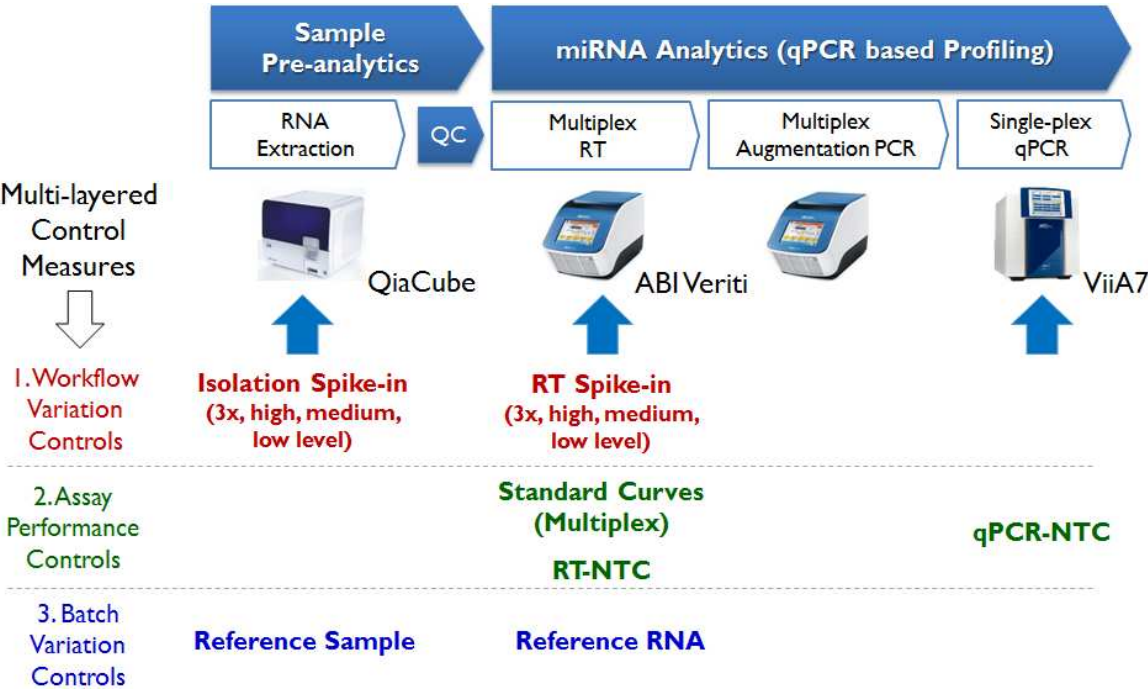


Figure S2. Analytical Validation of MIRXES miRNA RT-qPCR Assays. (A) Analytical Specificity Test. Table below shows the relative detection when assays are challenged with mismatched let-7 family members. **(B) Analytical Reproducibility Test.** Correlation of the expression profiles of 200 miRNAs quantified in 15 control and 15 gastric cancer sera in two independent laboratories ($R^2 > 0.95$) using MIRXES miRNA RT-qPCR assays. **(C) Analytical Sensitivity Test.** Comparison with Probe Based Assays. Consistency in the analytical sensitivity of the MIRXES assays was demonstrated by amplification and detection of a 7-log serial dilution of the synthetic templates of 8 miRNAs with low to high AT content. In contrast, probe based assays showed poorer consistency across miRNAs with different AT content.

Figure S2A

		Target	Assay relative detection							
			let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i
UGAGGUAG	UAGG	UUGUAUAGUU	let-7a	100.00%	0.00%	0.10%	1.20%	0.00%	0.50%	0.00%
UGAGGUAG	UAGG	UUGUGUGUU	let-7b	0.00%	100.00%	2.20%	0.00%	0.00%	0.00%	0.00%
UGAGGUAG	UAGG	UUGUAUGUU	let-7c	0.80%	1.60%	100.00%	0.00%	0.00%	0.00%	0.00%
UAGAGGUAG	UAGG	UUGCAUAGUU	let-7d	0.40%	0.00%	0.10%	100.00%	0.00%	0.00%	0.00%
UGAGGUAG	GAGG	UUGUAUAGUU	let-7e	0.10%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%
UGAGGUAG	UAGG	UUGUAUAGUU	let-7f	0.10%	0.00%	0.00%	0.00%	0.10%	100.00%	0.00%
UGAGGUAG	UAGG	UUGUAUAGUU	let-7g	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%
UGAGGUAG	UAGG	UUGUAUAGUU	let-7i	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
UGAGGUAG	UAGG	UUGUAUAGUU	miR-98	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%

Figure S2B

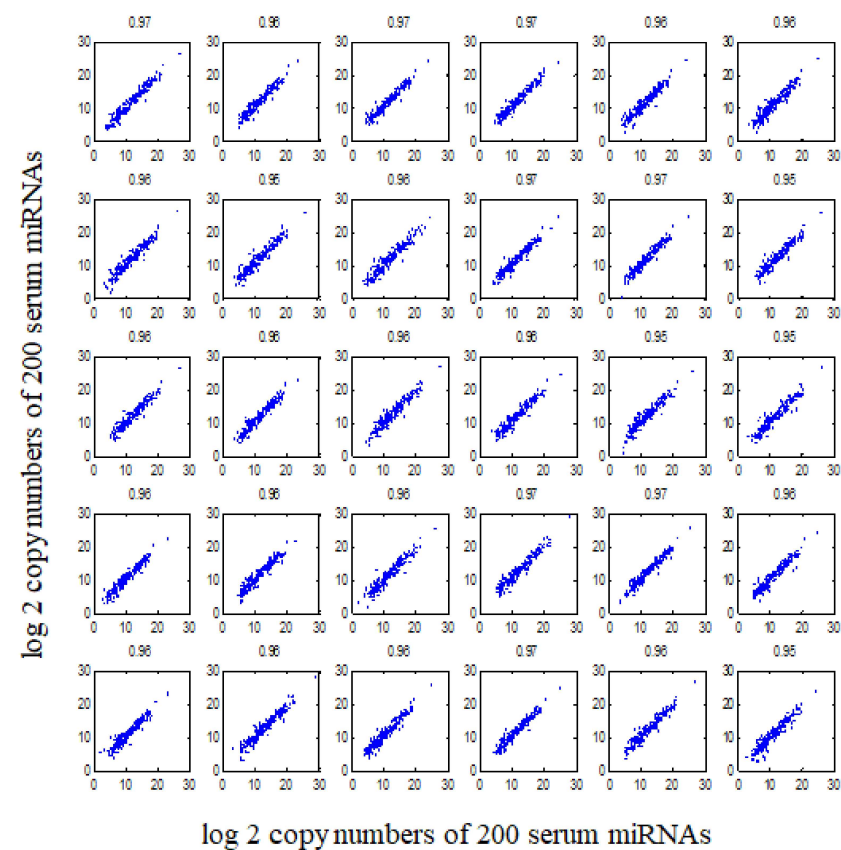


Figure S2C

#	miRNA	Sequence	AT%
1	hsa-miR-99b-5p	CACCCGUAGAACCGACCUUGCG	36.4%
2	hsa-miR-133a-3p	UUUGGUCCCCUUAACCAGCUG	45.5%
3	hsa-miR-593-3p	UGUCUCUGCUGGGGUUUCU	47.4%
4	hsa-miR-151a-5p	UCGAGGAGCUCACAGUCUAGU	47.6%
5	hsa-miR-20b-5p	CAAAGUGCUAUAGUGCAGGUAG	52.2%
6	hsa-miR-122-5p	UGGAGUGUGACAAUGGUGUUUG	54.5%
7	hsa-miR-377-3p	AUCACACAAAGGCAACUUUUGU	59.1%
8	hsa-miR-451a	AAACCGUUACCAUUACUGAGUU	63.6%

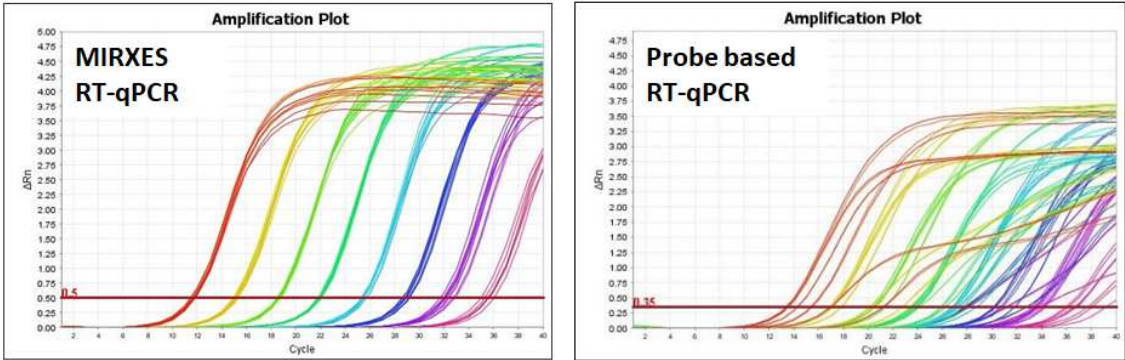


Figure S3. (A) Top up-regulated and down-regulated miRNAs in gastric cancer. The boxplots and ROC curves of top-ranked (based on AUC) up-regulated (miR-142-5p) and down-regulated (miR-99b-5p) miRNAs in all the gastric cancer subjects (regardless of subtypes and stages) compared to normal subjects; the expression levels (copy/ml) were presented in log2 scale. The boxplot presented the 25th, 50th, and 75th percentiles in the distribution of expression levels. AUC: area under the ROC curve. **(B) Top up-regulated and down-regulated miRNAs between normal and various stages of gastric cancers.** The ROC curves of top-ranking (based on AUC) up-regulated (row 1) and down-regulated (row 2) miRNAs in the various stages of gastric cancers subjects compared to the normal subjects. AUC: area under the ROC curve.

Figure S3A

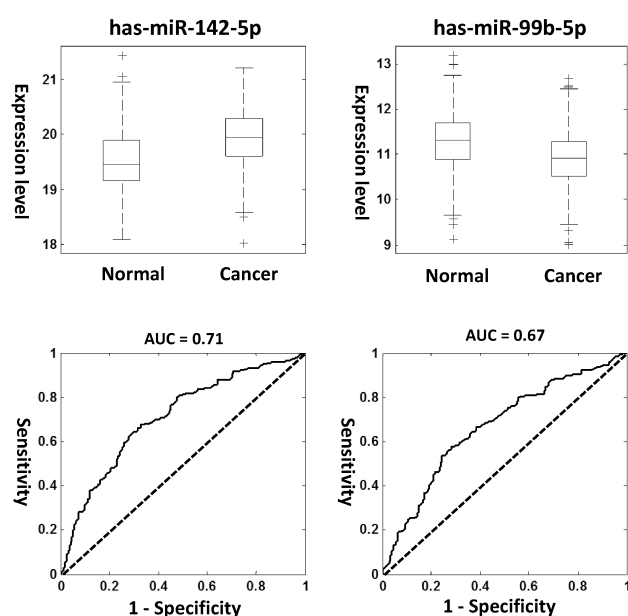


Figure S3B

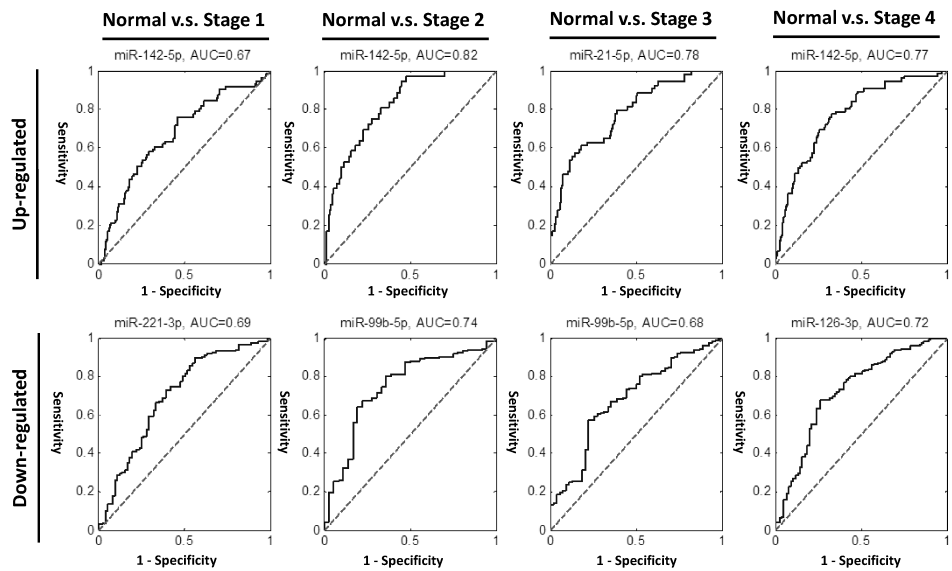


Figure S4. Differentially Expressed miRNAs between Various Gastric Cancer Subtypes. Boxplot of 7 miRNAs with p-values lower than 0.05 for three different subtypes based on two-way Anova test after false discovery rate (FDR) correction (Bonferroni method). The p-values were shown above the miRNAs. The boxplot presented the 25th, 50th, and 75th percentiles in the distribution of log2 scale expression levels (copy/ml). For each miRNA, the significant levels between various subtypes were calculated based on Bonferroni adjustment to compensate for multiple comparisons. *: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001. Five (hsa-miR-27a-3p, hsa-miR-338-5p, hsa-miR-181d, hsa-miR-146b-5p, hsa-miR-30e-3p) of these 7 miRNAs were found to be up-regulated and 2 were found to be down-regulated (hsa-miR-21-3p, hsa-miR-1226-3p) in the diffuse subtype when compared to the intestinal subtype. In addition, the expression level of these 7 miRNAs in the mixed subtype were found to be similar to the diffuse subtype except for hsa-miR-146b-5p, where the expression level in diffuse subtype was higher than the other two subtypes (middle lower panel).

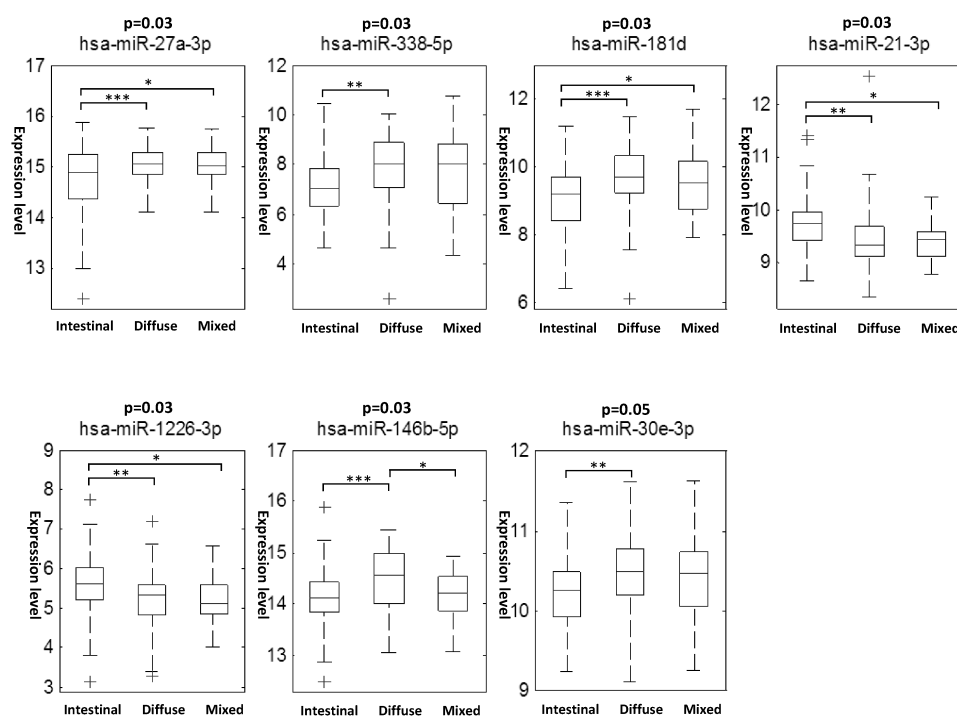


Figure S5. The impact of population compliance in screening program on early cancer diagnosis. With increasing compliance in the mass screening program, more cancer cases are expected to be diagnosed in early stages as compared to late stages. The figure below represents the expected pattern of early diagnosis with higher compliance rates. The ICER which is the cost spent to gain 1 additional QALY in the life of patient is independent of the compliance rate and is equivalent to USD 28,931/ QALY in all scenarios. *Abbreviations used- QALY: Quality-adjusted life years, ICER: Incremental cost-effectiveness ratio, Mn: Million, USD: United States Dollars*

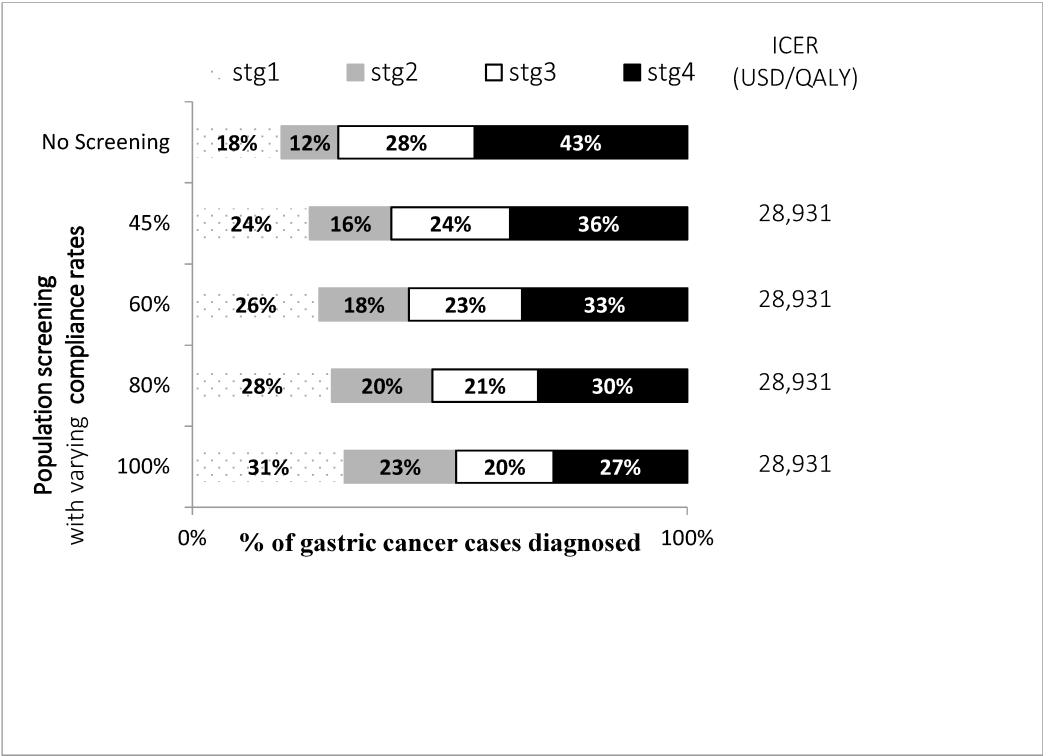


Figure S6. Comparison of Serum miRNA Biomarkers for Gastric, Breast and Ovarian Cancers. Three independent studies have been conducted to investigate serum miRNA expression changes between gastric, breast and ovarian cancers with their corresponding control populations. All measurements were performed using identical assays and workflows. The overlaps of up- and down-regulated serum miRNA biomarkers for these three cancers were presented in the venn diagram below. While there are some overlaps among the 3 cancers, distinct miRNAs changes specific to each cancer were observed. The heatmap below illustrates distinct serum miRNA expression in breast and gastric cancer patients.

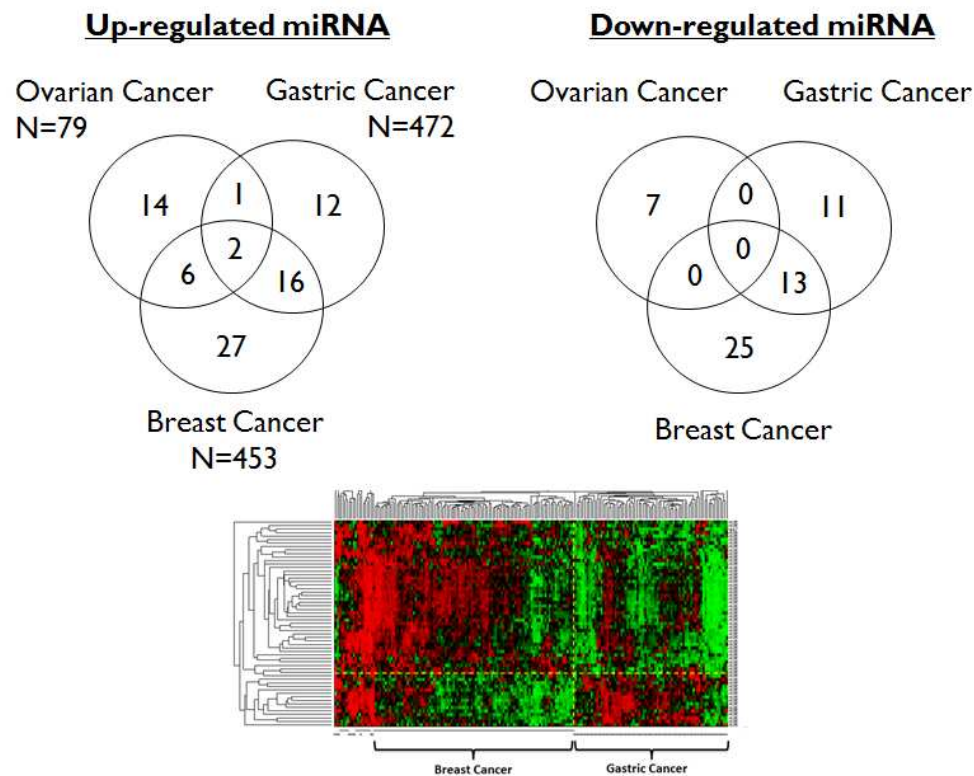


Figure S7. Markov-Decision Tree Model for evaluating the cost-effectiveness. The Markov decision model built has a 25-year time horizon and represents the movement of subjects from one health state to another in both the strategies. The patients who are diagnosed with cancer are treated as per the stage of diagnosis. Cancer recurrence is considered the only reason of treatment failure with all the recurrent cases being fatal. Subject is expected to exit the cohort at age 75. Health and cost parameters corresponding to each state are indicated at every step. The Markov model considers a 3% discount rate for both cost and health benefits and calculates values in their net present value.

Figure S7(A) Decision Tree Model for Strategy 1 – No screening. In current practice the subjects are diagnosed in clinic and treated as per the stage of diagnosis. The subjects in the population cohort would continue living healthy until being diagnosed as per the annual incidence rate reported in the Singapore cancer registry.

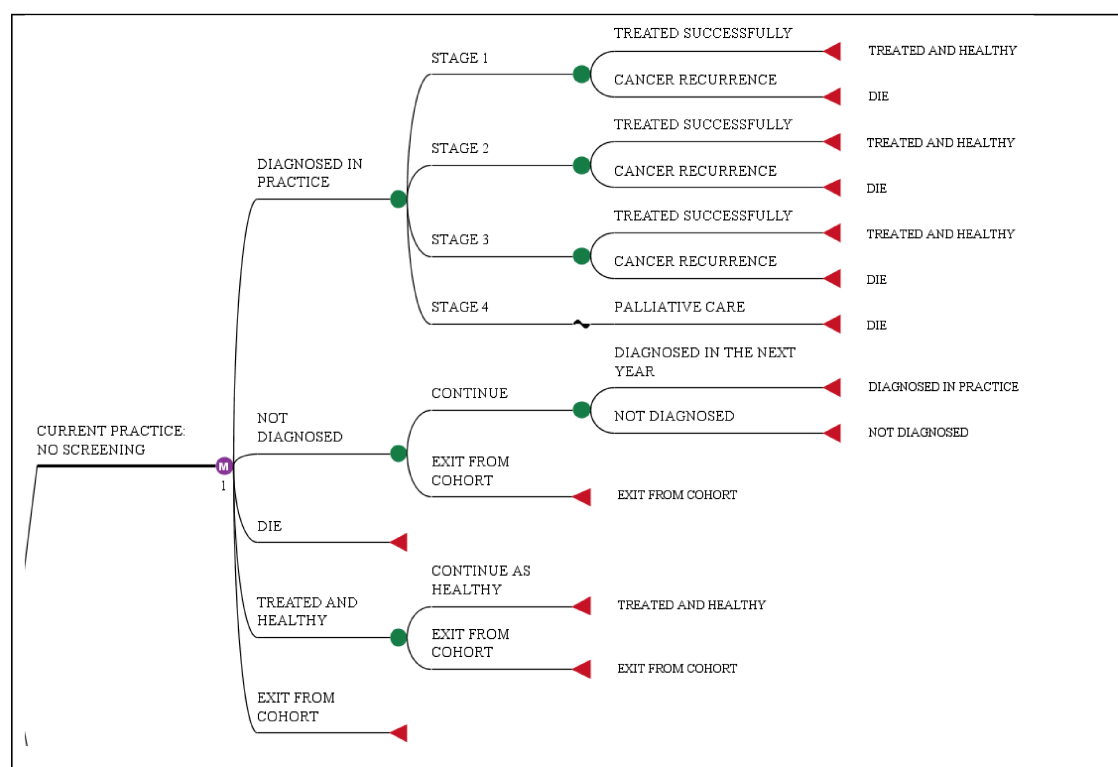


Figure S7(B) Decision Tree Model for Strategy2: mass screening with miRNA followed by endoscopy for diagnosis confirmation for test positive subjects and a 3-year follow up for test negative subjects. The subjects compliant to the mass screening program are made to undergo a miRNA-based blood test. If the miRNA test is positive, the subjects will undergo endoscopy and biopsy to confirm the cancer and if the miRNA test is negative, subjects are considered healthy until new cancers are diagnosed over time. Among this healthy group, there would be cancer cases missed by miRNA test. The missed cancer cases would progress to advanced stages and are expected to be diagnosed with symptoms at stage 4 in the clinic. The remaining healthy cases group is followed up with miRNA test every 3 years.

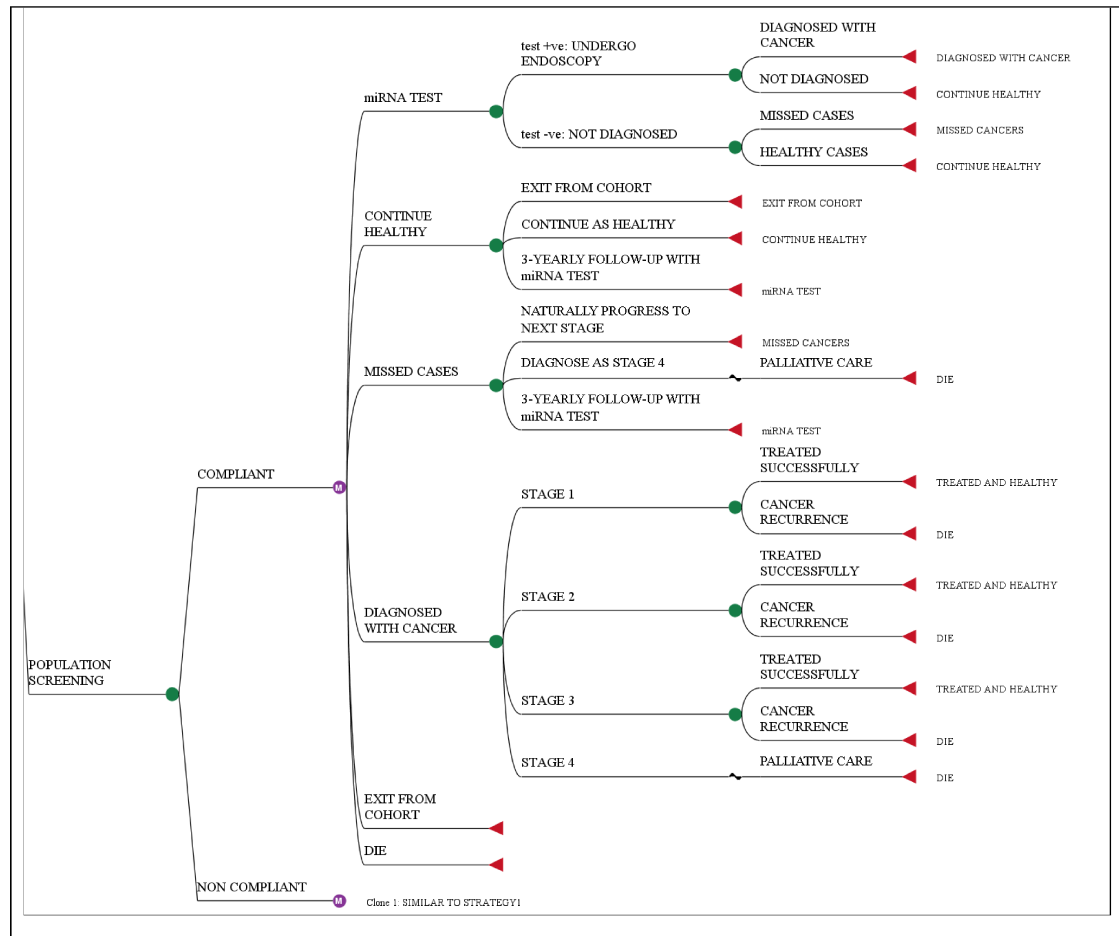


Figure S8. Sensitivity analysis for mass screening of Singaporean Chinese Males (50-75 years). We have performed one-way sensitivity analysis of many key variables to identify the impact of variable uncertainty on the Incremental Cost Effectiveness Ratio (ICER). The figure below shows all variables with their sensitivity ranges on Y axis and the ICER values on X axis. The range of values that were examined is shown in parentheses, with the value giving the lower ICER listed first. The graph represents the possible variation in ICER due to variable uncertainty, with the most significant variables at top. The solid vertical line indicates the ICER of 28,931 USD/QALY for the base-case scenario while the dash line indicates the threshold of ICER 50,000 USD/QALY. Three significant variables were identified which are: miRNA test cost, specificity of miRNA test and sensitivity of miRNA test for stage 1 patients. *Abbreviations used- QALY: Quality adjusted life years, ICER: Incremental cost-effectiveness ratio*

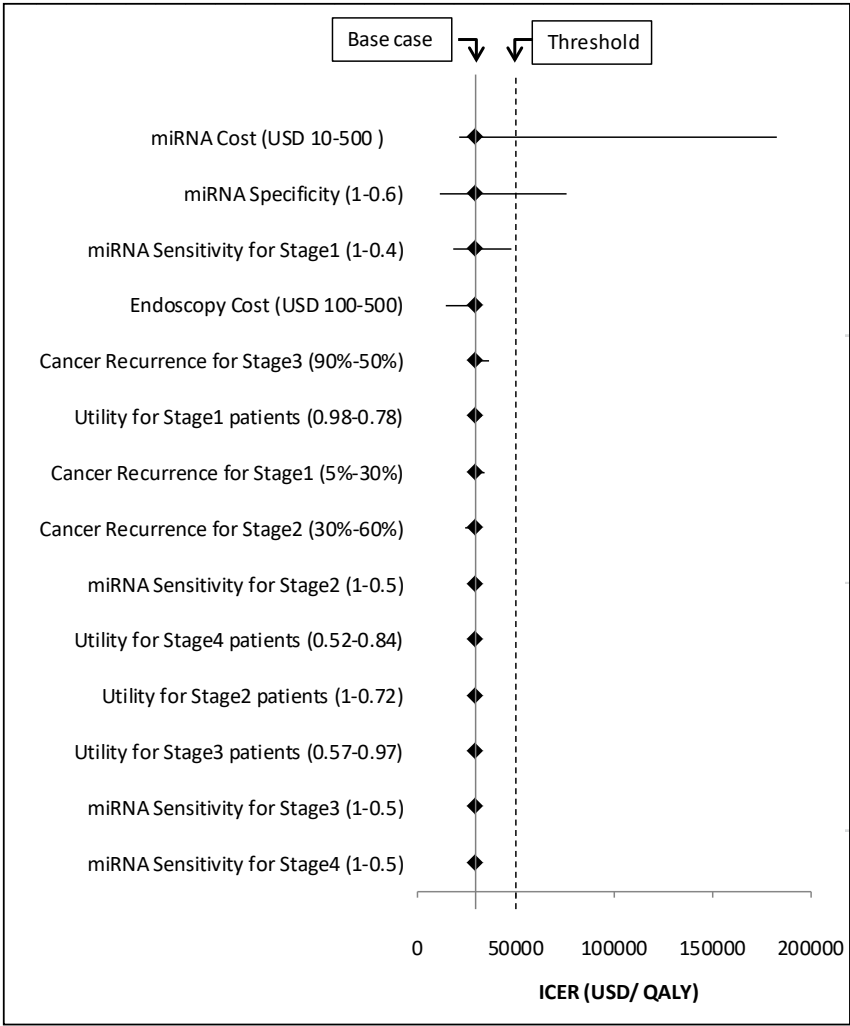


Figure S9. One-way sensitivity analysis for variables found significant for cost-effectiveness evaluation. The variation in ICER within the range of values of the three significant variables has been shown below individually. Also, their threshold value, i.e. value at which the ICER is equivalent to USD 50,000/QALY has been highlighted. The strategy would be cost-effective only with an ICER < 50,000 USD/QALY. For each sensitivity analysis below, it is assumed that the rest of variables remain constant as described in base-case scenario.

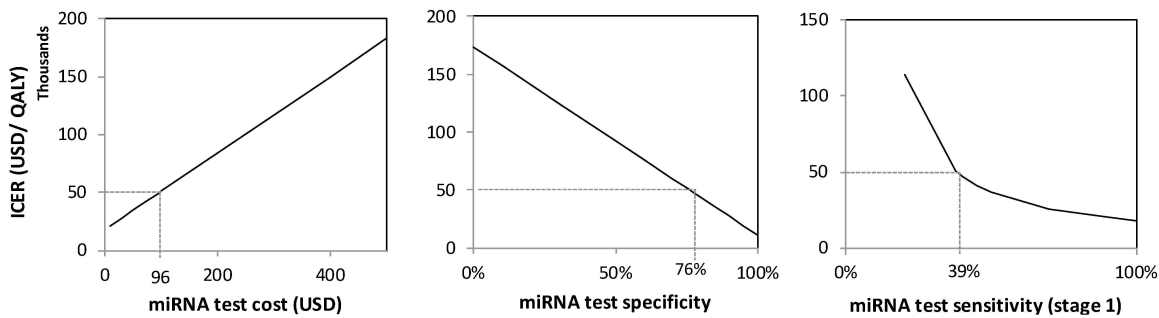


Figure S10. Sensitivity analysis of the cost-effectiveness of screening program with varying gastric cancer incidence. This sensitivity analysis has been performed to identify the cancer incidence which makes screening programs in the population cost-effective. The graph below reports the ICER at the different incidence rates ranging from 0.01% - 0.5%. The incidence rate reported is the average annual incidence for the target population. The analysis found the screening program to be cost-effective at an average incidence higher than 0.05% based on the cost-effectiveness threshold of US\$50,000/QALY. The target population of Singaporean Chinese males (50-75 years) is estimated to have an average annual gastric cancer incidence of 0.057% at the beginning of the analysis as per the 2016 population cohort statistics. With ageing, the average incidence rate of the cohort has been considered to increase.

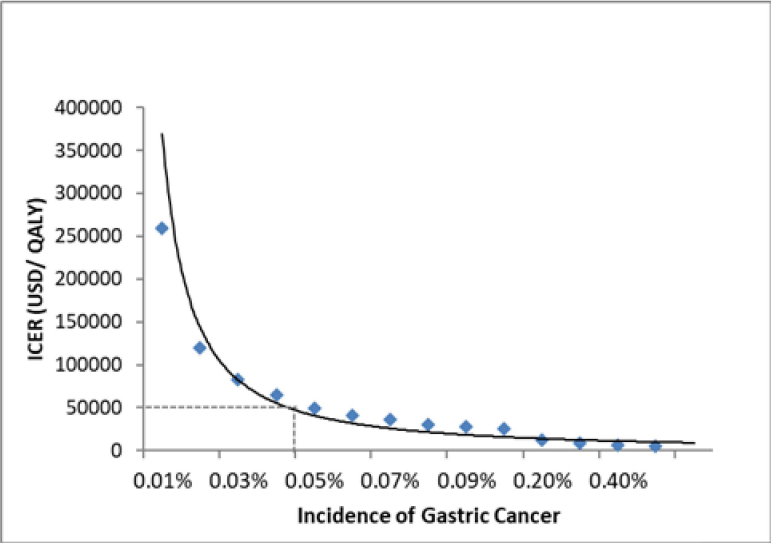


Table S1. Identity and Sequence of 191 Reliable Detected Mature miRNA. 191 mature miRNA were reliable detected in the serum samples. The definition of “reliably detected” was that at least 90% of the serum samples had a concentration higher than 500 copies per ml. The miRNAs were named according to the miRBase V18 release.

Identity	Sequence
hsa-miR-99b-5p	CACCCGUAGAACCGACCUUGCG
hsa-miR-486-5p	UCCUGUACUGAGCUGCCCCGAG
hsa-miR-23b-3p	AUCACAUUGCCAGGGAUUACC
hsa-miR-140-3p	UACCACAGGGUAGAACCACGG
hsa-miR-101-3p	UACAGUACUGUGAUAAACUGAA
hsa-miR-107	AGCAGCAUUGUACAGGGCUAUCA
hsa-miR-130b-3p	CAGUGCAAUGAUGAAAGGGCAU
hsa-miR-369-3p	AAUAAUACAUGGUUGAUCUUU
hsa-miR-133a	UUUGGUCCCCUUAACCAGCUG
hsa-miR-222-3p	AGCUACAUCUGGCUACUGGGU
hsa-miR-320d	AAAAGCUGGGUUGAGAGGA
hsa-miR-30a-5p	UGUAAACAUCUCGACUGGAAG
hsa-miR-181a-5p	AACAUUCAACGCUGUCGGUGAGU
hsa-miR-140-5p	CAGUGGUUUUACCCUAUGGUAG
hsa-miR-425-3p	AUCGGGAUGUCGUGUCCGCC
hsa-miR-106b-3p	CCGCACUGUGGGUACUUGCUGC
hsa-miR-192-5p	CUGACCUAUGAAUUGACAGCC
hsa-miR-10a-3p	CAAAUUCGUAUCUAGGGGAAUA
hsa-miR-17-5p	CAAAGUCUACAGUGCAGGUAG
hsa-miR-590-5p	GAGCUUAUUAUAAAAGUGCAG
hsa-miR-1299	UUCUGGAAUUCUGUGUGAGGGA
hsa-miR-365a-3p	UAAUGCCCCUAAAAUCCUUUAU
hsa-miR-500a-5p	UAAUCCUUGCUACCUGGGUGAGA
hsa-miR-32-5p	UAUUGCACAUUACUAAGUUGCA
hsa-miR-340-5p	UUUAAAAGCAAUGAGACUGAUU
hsa-miR-374b-5p	AUAUAAUACAACCUGCUAAGUG
hsa-miR-27a-3p	UUCACAGUGGCUAAGUUCGCG
hsa-miR-627	GUGAGUCUCUAAGAAAAGAGGA
hsa-miR-539-5p	GGAGAAUUAUCCUUGGUGUGU
hsa-miR-342-5p	AGGGGUGCUAUCUGUGAUUGA
hsa-miR-484	UCAGGCUCAGUCCCCUCCCGAU
hsa-miR-132-3p	UACAGUCUACAGCCAUGGUCG
hsa-miR-379-5p	UGGUAGACUAUGGAACGUAGG
hsa-miR-125a-3p	ACAGGUGAGGUUCUUGGGAGCC
hsa-miR-29a-3p	UAGCACCAUCUGAAAUCGGUUA

hsa-miR-363-3p	AAUUGCACGGUAUCCAUCUGUA
hsa-miR-376b	AUCAUAGAGGAAAAUCCAUGUU
hsa-miR-589-5p	UGAGAACCACGUCUGCUCUGAG
hsa-miR-432-5p	UCUUGGAGUAGGUCAUUGGGUGG
hsa-miR-1280	UCCCACCGCUGCCACCC
hsa-miR-103a-3p	AGCAGCAUUGUACAGGGCUAUGA
hsa-miR-122-5p	UGGAGUGUGACAAUGGUGUUUG
hsa-miR-93-5p	CAAAGUGCUGUUCGUGCAGGUAG
hsa-miR-25-3p	CAUUGCACUUGUCUCGGUCUGA
hsa-miR-9-5p	UCUUUGGUUAUCUAGCUGUAUGA
hsa-miR-579	UUCAUUUGGUUAAAACCGCGAUU
hsa-miR-136-3p	CAUCAUCGUCUCAAUGAGUCU
hsa-miR-146a-5p	UGAGAACUGAAUCCAUGGGUU
hsa-miR-144-5p	GAUAUCAUCAUAUACUGUAAG
hsa-miR-15a-5p	UAGCAGCACAUAAUGGUUUUGUG
hsa-miR-150-5p	UCUCCCAACCCUUGUACCAGUG
hsa-miR-152	UCAGUGCAUGACAGAACUUGG
hsa-miR-29c-5p	UGACCGAUUUCUCCUGGUGUUC
hsa-miR-320c	AAAAGCUGGGUUGAGAGGGU
hsa-miR-127-3p	UCGGAUCCGUCUGAGCUUGGCU
hsa-miR-331-5p	CUAGGUAUGGUCCCAGGGAUCC
hsa-miR-378a-3p	ACUGGACUUGGAGUCAGAAGG
hsa-miR-374a-5p	UUUAUAUACAACCUGAUAAUGUG
hsa-miR-409-3p	GAAUGUUGCUCGGUGAACCCCU
hsa-miR-411-3p	UAUGUAACACGGUCCACUAACC
hsa-miR-505-3p	CGUCAACACUUGCUGGUUUCCU
hsa-miR-628-5p	AUGCUGACAUAUUUACUAGAGG
hsa-miR-629-3p	GUUCUCCCAACGUAAGCCCAGC
hsa-miR-4732-3p	GCCCUGACCUGUCCUGUUCUG
hsa-miR-501-5p	AAUCCUUUGUCCUGGGUGAGA
hsa-miR-616-5p	ACUCAAACCCUUCAGUGACUU
hsa-miR-454-3p	UAGUGCAAUAUUGCUUAUAGGGU
hsa-miR-485-3p	GUCAUACACGGCUCUCCUCUCU
hsa-miR-133b	UUUGGUCCCCUUAACCAGCUA
hsa-miR-186-5p	CAAAGAAUUCUCCUUUUGGGCU
hsa-miR-20b-5p	CAAAGUGCUCAUAGUGCAGGUAG
hsa-miR-30d-5p	UGUAAACAUCCCCGACUGGAAG
hsa-miR-375	UUUGUUCGUUCGGCUCGCGUGA
hsa-miR-16-5p	UAGCAGCACGUAAAUUUGGCG
hsa-miR-106b-5p	UAAAGUGCUGACAGUGCAGAU
hsa-miR-139-5p	UCUACAGUGCACGUGUCUCCAG

hsa-miR-141-3p	UAACACUGUCUGGUAAGAUGG
hsa-miR-185-5p	UGGAGAGAAAGGCAGUCCUGA
hsa-miR-181b-5p	AACAUUCAUUGCUGUCGGUGGGU
hsa-miR-199a-3p	ACAGUAGUCUGCACAUUGGUUA
hsa-miR-19b-3p	UGUGCAAAUCCAUGCAAAACUGA
hsa-miR-148b-3p	UCAGUGCAUCACAGAACUUUGU
hsa-miR-29b-3p	UAGCACCAUUUGAAAUCAGUGUU
hsa-miR-338-5p	AACAAUAUCCUGGUGCUGAGUG
hsa-miR-584-5p	UUAUGGUUUGCCUGGGACUGAG
hsa-miR-382-5p	GAAGUUGUUCGUGGUGGAUUCG
hsa-miR-151a-3p	CUAGACUGAAGCUCCUUGAGG
hsa-miR-1290	UGGAUUUUUGGAUCAGGGA
hsa-miR-200b-3p	UAAUACUGCCUGGUAUGAUGA
hsa-miR-411-5p	UAGUAGACCGUAUAGCGUACG
hsa-miR-126-5p	CAUUUUUACUUUUGGUACGCG
hsa-miR-101-5p	CAGUUAUCACAGUGCUGAUGCU
hsa-miR-125b-5p	UCCCUGAGACCCUAAUUGUGA
hsa-miR-362-5p	AAUCCUUGGAACCUAGGUGUGAGU
hsa-miR-197-3p	UUCACCACCUUCCUCCACCCAGC
hsa-miR-221-3p	AGCUACAUUGUCUGCUGGGUUUC
hsa-miR-501-3p	AAUGCACCCGGGCAAGGAUUCU
hsa-miR-671-3p	UCCGGUUCUCAGGGCUCCACC
hsa-miR-181a-2-3p	ACCACUGACCGUUGACUGUACC
hsa-miR-9-3p	AUAAAGCUAGAUAAACCGAAAGU
hsa-miR-452-5p	AACUGUUUGCAGAGGAAACUGA
hsa-miR-598	UACGUCAUCGUUGUCAUCGUCA
hsa-miR-320b	AAAAGCUGGGUUGAGAGGGCAA
hsa-miR-328	CUGGCCCUCUCUGCCCUUCCGU
hsa-miR-650	AGGAGGCAGCGCUCUCAGGAC
hsa-miR-134	UGUGACUGGUUGACCAGAGGGG
hsa-miR-130a-3p	CAGUGCAAUGUUAAAAGGGCAU
hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-424-5p	CAGCAGCAAUUCAUUUUUGAA
hsa-miR-99a-5p	AACCCGUAGAUCCGAUCUUGUG
hsa-miR-18a-3p	ACUGCCCUAAGUGCUCCUUCUGG
hsa-miR-195-5p	UAGCAGCACAGAAAUUUUGGC
hsa-miR-205-5p	UCCUUCAUUCCACCGGAGUCUG
hsa-miR-206	UGGAAUGUAAGGAAGUGUGUGG
hsa-miR-500a-3p	AUGCACCUGGGCAAGGAUUCUG
hsa-miR-18b-5p	UAAGGUGCAUCUAGUGCAGUUAG
hsa-miR-181d	AACAUUCAUUGUUGUCGGUGGGU

hsa-miR-339-3p	UGAGCGCCUCGACGACAGAGCCG
hsa-miR-93-3p	ACUGCUGAGCUAGCACUUCGCCG
hsa-miR-10b-5p	UACCCUGUAGAACCGAAUUUGUG
hsa-miR-497-5p	CAGCAGCACACUGUGGUUUUGU
hsa-miR-27b-3p	UUCACAGUGGCUAAGUUCUGC
hsa-miR-128	UCACAGUGAACCGGUCUCUUU
hsa-miR-183-5p	UAUGGCACUGGUAGAAUUCACU
hsa-miR-22-3p	AAGCUGCCAGUUGAAGAACUGU
hsa-miR-26a-5p	UUCAAGUAAUCCAGGAUAGGCU
hsa-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA
hsa-miR-629-5p	UGGGUUUACGUUGGGGAGAACU
hsa-miR-92a-3p	UAUUGCACUUGUCCCGGCCUGU
hsa-miR-29b-2-5p	CUGGUUUCACAUGGUGGCUUAG
hsa-miR-21-3p	CAACACCAGUCGAUGGGCUGU
hsa-miR-199a-5p	CCCAGUGUUCAGACUACCUGUUC
hsa-miR-148a-3p	UCAGUGCACUACAGAACUUUGU
hsa-miR-193a-5p	UGGGUCUUUGCGGGCGAGAUGA
hsa-miR-27a-5p	AGGGCUUAGCUGCUUGUGAGCA
hsa-miR-200c-3p	UAAUACUGCCGGGUAAUGAUGGA
hsa-miR-20a-5p	UAAAGUGCUUAUAGUGCAGGUAG
hsa-miR-194-5p	UGUAAACAGCAACUCCAUGUGGA
hsa-miR-532-3p	CCUCCCACACCCAAGGCUUGCA
hsa-miR-19a-3p	UGUGCAAAUCUAUGCAAAACUGA
hsa-miR-142-5p	CAUAAAGUAGAAAGCACUACU
hsa-miR-144-3p	UACAGUAUAGAUGAUGUACU
hsa-miR-145-5p	GUCCAGUUUUCCCAGGAUCCCU
hsa-miR-10a-5p	UACCCUGUAGAUCCGAUUUUGUG
hsa-miR-23a-3p	AUCACAUUGCCAGGGAUUUCC
hsa-miR-23a-5p	GGGGUUCCUGGGGAUGGGAUUU
hsa-miR-15b-3p	CGAAUCAUUAUUUGCUGCUCUA
hsa-miR-301a-3p	CAGUGCAAUAGUAUUGUCAAAAGC
hsa-miR-660-5p	UACCCAUUGCAUAUCGGAGUUG
hsa-miR-30b-5p	UGUAAACAUCUACACUCAGCU
hsa-miR-30e-5p	UGUAAACAUCUUGACUGGAAG
hsa-miR-550a-5p	AGUGCCUGAGGGAGUAAGAGCCC
hsa-miR-425-5p	AAUGACACGAUCACUCCCGUUGA
hsa-miR-4306	UGGAGAGAAAGGCAGUA
hsa-miR-532-5p	CAUGCCUUGAGUGUAGGACCGU
hsa-miR-335-5p	UCAAGAGCAAUACGAAAAAUGU
hsa-miR-483-5p	AAGACGGGAGGAAAGAAGGGAG
hsa-miR-1226-3p	UCACCAGCCCUGUGUUCCCUAG

hsa-miR-431-5p	UGUCUUGCAGGCCGUGAUGCA
hsa-miR-324-5p	CGCAUCCCCUAGGGCAUUGGUGU
hsa-miR-487b	AAUCGUACAGGGUCAUCCACUU
hsa-miR-451a	AAACCGUUACCAUACUGAGUU
hsa-miR-493-5p	UUGUACAUGGUAGGCUUUCUU
hsa-miR-136-5p	ACUCCAUUUUUUGAUGAUGGA
hsa-miR-23c	AUCACAUUGCCAGUGAUUACCC
hsa-miR-95	UUCAACGGGUUUUUUUGAGCA
hsa-miR-423-5p	UGAGGGGCAGAGAGCGAGACUUU
hsa-miR-320e	AAAGCUGGGUUGAGAAGG
hsa-miR-224-5p	CAAGUCACUAGUGGUUCCGUU
hsa-miR-28-3p	CACUAGAUUGUGAGCUCCUGGA
hsa-miR-29c-3p	UAGCACCAUUUGAAAUCGGUUA
hsa-miR-326	CCUCUGGGCCCUUCCUCCAG
hsa-miR-596	AAGCCUGCCCGGCUCCUCGGG
hsa-miR-885-5p	UCCAUUACACUACCCUGCCUCU
hsa-miR-146b-5p	UGAGAACUGAAUCCAUAGGCU
hsa-miR-34a-5p	UGGCAGUGUCUUAGCUGGUUGU
hsa-miR-330-3p	GCAAAGCACACGGCCUGCAGAGA
hsa-miR-154-5p	UAGGUUAUCCGUGUUGCCUUCG
hsa-miR-191-5p	CAACGGAAUCCCAAAAGCAGCUG
hsa-miR-193b-3p	AACUGGCCCUCAAAGUCCGCU
hsa-miR-301b	CAGUGCAAUGAUUUUGUCAAAGC
hsa-miR-30e-3p	CUUUCAGUCGGAUGUUUACAGC
hsa-miR-320a	AAAAGCUGGGUUGAGAGGGCGA
hsa-miR-199b-3p	ACAGUAGUCUGCACAUUGGUUA
hsa-miR-502-3p	AAUGCACCUGGGCAAGGAUUA
hsa-miR-450a-5p	UUUUGCGAUGUGUUCUAAUUAU
hsa-miR-495	AAACAAACAUGGUGCACUUCUU
hsa-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG
hsa-miR-15b-5p	UAGCAGCACAUCAUGGUUUACA
hsa-miR-339-5p	UCCCUGUCCUCCAGGAGCUCACG
hsa-miR-337-5p	GAACGGCUUCAUACAGGAGUU

Table S2. miRNAs Differentially Expressed between Normal and Gastric Cancer. For the comparison between normal and all gastric cancer subjects (regardless of subtypes and stages), 75 miRNA had p-value lower than 0.01 after FDR correction (Bonferroni method). AUC – area under the receiver operating characteristic curve; fold change – the mean expression level (copy/ml) of miRNA in the cancer population divided by that in the normal population.

Up-regulated miRNAs

miRNA name	AUC	P-value	P-value, FDR correction	Fold change	Novel Observation
miR-101-3p	0.61	1.80E-05	9.50E-05	1.27	Novel
miR-106b-3p	0.66	3.70E-09	4.10E-08	1.13	Novel
miR-106b-5p	0.61	5.10E-04	1.70E-03	1.21	
miR-128	0.62	1.40E-06	8.80E-06	1.16	Novel
miR-1280	0.66	3.10E-09	3.90E-08	1.38	Novel
miR-140-3p	0.62	6.40E-06	3.50E-05	1.2	Novel
miR-140-5p	0.67	6.20E-10	1.20E-08	1.24	Novel
miR-142-5p	0.71	1.90E-14	3.70E-12	1.31	Novel
miR-148a-3p	0.67	2.20E-10	4.80E-09	1.32	Novel
miR-15b-3p	0.62	6.20E-06	3.50E-05	1.32	Novel
miR-17-5p	0.63	1.00E-05	5.50E-05	1.24	
miR-183-5p	0.64	8.80E-07	6.20E-06	1.53	Novel
miR-186-5p	0.59	1.40E-03	3.70E-03	1.11	Novel
miR-18b-5p	0.64	1.50E-07	1.20E-06	1.38	Novel
miR-197-3p	0.68	8.10E-13	5.10E-11	1.32	Novel
miR-19a-3p	0.63	4.90E-07	3.60E-06	1.29	Novel
miR-19b-3p	0.59	1.10E-03	3.00E-03	1.18	Novel
miR-20a-5p	0.65	1.20E-07	1.10E-06	1.35	
miR-20b-5p	0.60	2.90E-04	9.90E-04	1.3	Novel
miR-21-3p	0.60	7.90E-05	3.20E-04	1.13	Novel
miR-21-5p	0.63	2.60E-08	2.80E-07	1.23	
miR-223-3p	0.66	7.00E-10	1.20E-08	1.36	
miR-23a-5p	0.64	1.00E-07	9.20E-07	1.31	Novel
miR-25-3p	0.62	3.40E-05	1.60E-04	1.26	Novel
miR-27a-5p	0.69	1.00E-13	1.00E-11	1.76	
miR-29a-3p	0.61	6.00E-05	2.60E-04	1.17	Novel
miR-29b-2-5p	0.59	4.70E-05	2.10E-04	1.16	Novel
miR-29b-3p	0.61	7.20E-05	3.00E-04	1.18	Novel
miR-29c-3p	0.65	2.00E-09	2.90E-08	1.23	Novel
miR-29c-5p	0.63	1.40E-06	8.80E-06	1.15	Novel
miR-338-5p	0.57	3.70E-03	9.40E-03	1.29	Novel
miR-423-5p	0.60	7.20E-05	3.00E-04	1.18	
miR-424-5p	0.68	7.00E-11	1.90E-09	1.41	Novel

miR-425-3p	0.57	2.20E-03	5.70E-03	1.05	Novel
miR-4306	0.63	1.20E-06	8.00E-06	1.35	Novel
miR-450a-5p	0.67	2.10E-10	4.80E-09	1.53	Novel
miR-486-5p	0.61	9.60E-05	3.70E-04	1.32	Novel
miR-500a-3p	0.60	1.10E-04	4.20E-04	1.2	Novel
miR-501-5p	0.60	9.60E-04	2.80E-03	1.24	Novel
miR-532-3p	0.60	1.90E-04	7.00E-04	1.15	Novel
miR-550a-5p	0.63	9.00E-07	6.20E-06	1.38	Novel
miR-579	0.62	2.20E-05	1.10E-04	1.3	Novel
miR-589-5p	0.63	1.70E-06	1.00E-05	1.18	Novel
miR-590-5p	0.69	3.00E-12	1.40E-10	1.23	Novel
miR-598	0.67	7.10E-12	2.70E-10	1.27	Novel
miR-616-5p	0.65	3.40E-09	4.10E-08	1.35	Novel
miR-627	0.58	7.30E-04	2.30E-03	1.19	Novel
miR-629-3p	0.67	6.10E-11	1.90E-09	1.38	Novel
miR-629-5p	0.63	1.40E-04	5.10E-04	1.5	Novel
miR-93-3p	0.62	5.10E-06	3.00E-05	1.22	Novel
miR-93-5p	0.60	2.30E-04	8.00E-04	1.21	Novel

Down-regulated miRNAs

miRNA name	AUC	P-value	P-value, FDR correction	Fold change	
miR-107	0.65	4.40E-08	4.40E-07	0.8	Novel
miR-122-5p	0.61	8.10E-05	3.20E-04	0.66	Novel
miR-126-3p	0.66	1.70E-09	2.70E-08	0.87	Novel
miR-136-5p	0.61	2.30E-05	1.10E-04	0.72	Novel
miR-139-5p	0.60	8.60E-05	3.40E-04	0.84	Novel
miR-146a-5p	0.59	2.10E-03	5.60E-03	0.89	Novel
miR-154-5p	0.59	8.60E-04	2.60E-03	0.8	Novel
miR-181a-5p	0.60	2.30E-04	8.00E-04	0.92	Novel
miR-193b-3p	0.58	1.20E-03	3.20E-03	0.77	Novel
miR-23c	0.59	8.00E-04	2.40E-03	0.84	Novel
miR-26a-5p	0.60	4.40E-05	2.00E-04	0.86	Novel
miR-30a-5p	0.64	6.70E-08	6.40E-07	0.76	Novel
miR-30b-5p	0.59	9.50E-04	2.80E-03	0.9	Novel
miR-337-5p	0.63	4.80E-07	3.60E-06	0.74	Novel
miR-339-5p	0.64	4.90E-07	3.60E-06	0.79	Novel
miR-382-5p	0.59	1.00E-03	2.90E-03	0.81	Novel

miR-409-3p	0.59	5.00E-04	1.60E-03	0.77	Novel
miR-411-5p	0.6	7.30E-04	2.30E-03	0.74	Novel
miR-485-3p	0.6	6.40E-04	2.00E-03	0.77	Novel
miR-487b	0.59	1.10E-03	3.00E-03	0.76	Novel
miR-495	0.6	2.10E-04	7.40E-04	0.77	Novel
miR-885-5p	0.62	1.90E-05	9.60E-05	0.69	Novel
miR-99a-5p	0.58	2.90E-03	7.50E-03	0.82	Novel
miR-99b-5p	0.67	2.60E-09	3.50E-08	0.78	Novel

Table S3. Summary of Serum / Plasma miRNA Biomarker Studies for Gastric Cancer. The studies that measured the cell-free serum/plasma miRNAs were included in the table. Only the results validated with RT-qPCR were shown. GC: gastric cancer subjects. C: control subjects.

Paper	Up regulated	Down regulated	Method	Samples
Chen Li et al [13]	miR-199a-3p	-	RT-qPCR	Plasma/80GC/70C
Aysegul Gorur et al [14]	-	miR-195-5p	RT-qPCR	Serum/20GC/190C
Hui Cai et al [15]	miR-106b, miR-20a, miR-221	-	RT-qPCR	Plasma/90GC/90C
Mei-Hua Cui et al [16]	miR-181c	-	RT-qPCR	Plasma/30GC/60C
Chen Li et al [17]	miR-199a-3p, miR-151-5p	-	RT-qPCR	Plasma/180GC/100C
Ming-yang Song et al [18]	miR-221, miR-744, miR-376c, miR-191, miR-27a, let-7e, miR-27b, and miR-222	-	RT-qPCR	Serum/82GC/82C
Bo-sheng Li et al [19]	miR-223, miR-21	miR- 218	RT-qPCR	Plasma/60GC/60C
Manuel Valladares-Ayerbes et al [20]	miR-200c	-	RT-qPCR	whole blood/52GC/15C
Wen-Hui Zhang et al [21]	-	miR-375	RT-qPCR	Serum
S. S. Lo et al [22]	miR-370	-	RT-qPCR	Plasma/33GC/33C
M. Tsujiura et al [23]	miR-17-5p, miR-21, miR-106a, miR-106b	let-7a	RT-qPCR	Plasma/69GC/30C
Rui Liu et al [24]	miR-1, miR-20a, miR-27a, miR-34a, miR-423-5p	-	RT-qPCR	Serum/142GC/105C
Hanshao Liu et al [25]	miR-187*,miR-371-5p, miR-378	-	RT-qPCR	Serum/40GC/41C

Table S4. MiRNAs Differentially Expressed between Different Stages of Gastric Cancer. A total of 36 miRNAs with p-value lower than 0.05 were identified from the comparison made with the four stages of gastric cancer, based on two-way anova test (subtypes and stages) after false discovery rate correction (Bonferroni method). The expression levels (copy/ml) were analyzed based on the log2 scale. For each miRNA, the significant levels for the alternations between stage 1 and 2, stage 2 and 3, stage 3 and 4 were calculated based on anova test and Bonferroni adjustment to compensate for multiple comparisons. *: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001. A miRNA was considered up-regulated if its expression level was higher in the later stage.

	anova p<0.01	Group	change between stage 2 and stage 1	change between stage 3 and stage 2	change between stage 4 and stage 3	change between normal and all cancer
hsa-miR-27a-3p	0.0008	I	-	down**	up***	No change
hsa-miR-1280	0.0007	C	up***	-	-	up-regulated
hsa-miR-579	0.0067	A	-	-	-	up-regulated
hsa-miR-150-5p	0.0035	F	-	-	-	No change
hsa-miR-29c-5p	0.0010	B	up***	-	-	up-regulated
hsa-miR-186-5p	0.0087	H	up**	down**	-	up-regulated
hsa-miR-338-5p	0.0072	L	-	-	up**	up-regulated
hsa-miR-362-5p	0.0014	B	up**	-	-	No change
hsa-miR-197-3p	0.0000	B	up***	-	-	up-regulated
hsa-miR-221-3p	0.0000	B	up***	-	-	No change
hsa-miR-501-3p	0.0000	C	up***	-	down*	No change
hsa-miR-181a- 2-3p	0.0072	G	up**	-	-	No change
hsa-miR-598	0.0000	A	up**	-	-	up-regulated
hsa-miR-320b	0.0014	C	up**	-	-	No change
hsa-miR-328	0.0000	C	up***	-	down*	No change
hsa-miR-134	0.0072	D	-	-	down***	No change
hsa-miR-21-5p	0.0000	E	-	up**	down**	up-regulated
hsa-miR-424-5p	0.0000	B	up***	-	-	up-regulated
hsa-miR-99a-5p	0.0023	G	up*	-	down**	down- regulated
hsa-miR-18a-3p	0.0016	B	up***	-	-	No change
hsa-miR-195-5p	0.0000	G	up***	-	down**	No change
hsa-miR-500a- 3p	0.0000	C	up***	-	down**	up-regulated
hsa-miR-18b-5p	0.0072	C	up*	-	-	up-regulated
hsa-miR-339-3p	0.0005	C	up***	-	-	No change
hsa-miR-128	0.0000	C	up***	-	down***	up-regulated
hsa-miR-22-3p	0.0016	C	-	-	down**	No change
hsa-miR-26a-5p	0.0002	G	up**	-	down***	down- regulated
hsa-miR-29b-2- 5p	0.0087	B	up*	-	-	up-regulated

hsa-miR-148a-3p	0.0029	A	-	-	-	up-regulated
hsa-miR-142-5p	0.0004	H	up**	down***	up**	up-regulated
hsa-miR-23a-3p	0.0000	B	up***	-	-	No change
hsa-miR-23c	0.0002	C	up**	-	down**	down-regulated
hsa-miR-28-3p	0.0072	K	down*	-	-	No change
hsa-miR-193b-3p	0.0029	K	down**	-	-	down-regulated
hsa-miR-320a	0.0004	J	down***	up**	-	No change
hsa-miR-15b-5p	0.0000	B	up***	down*	-	No change

Table S5. Multivariate Cox regression analysis of 12-miRNA panel and clinical covariates

Variables		Log Hazard Ratio, ln(HR)	p-value
12-miR	Cancer vs Non-cancer	13.9	< 0.001
Age (years)	> 50 vs ≤ 50	0.66	0.04
Gender	Male vs Female	0.54	0.47
Ethnicity	Chinese vs Non-Chinese	0.17	0.64
<i>H. pylori</i>	Yes vs No	-0.04	0.89

Table S6. Cross-reactivity test against other common cancers

#	Type of Cancer	Number of specimens tested	Number of specimen with high risk score based on GASTROClear
1	Esophageal	12	1
2	Liver	6	1
3	Colorectal	12	3
4	Lung	12	1
5	Breast	12	0
6	Prostate	12	0
7	Kidney	12	5
8	Bladder	12	0
	Total	90	11

Table S7. Base-case Values and Corresponding Sensitivity Range for Variables in Cost-Effectiveness Modelling

Singaporean healthcare setup			
Variable name	Base-case value	Sensitivity Range	Source
Costs (USD)			
MiRNA test	30	10 – 500	Assumed
Upper -endoscopy (EGD)	493	100 - 500	National University Hospital, Singapore (NUH)
Biopsy	122	-	
Stage 1 treatment	10423	-	
Stage 2 treatment	10423	-	
Stage 3 treatment	29451	-	
Stage 4 treatment	3069	-	
Follow-up examinations	719	-	
Staging Investigation (EUS + CT+ CXR+ follow-up)	1513	-	
Probabilities			
Incidence of Gastric Cancer in Chinese Males by Age group			Report No.8, 2015. Singapore Cancer Registry [26]
50 - 54 years	0.018%		
55 - 59 years	0.029%		
60 - 64 years	0.053%		
65 - 69 years	0.098%		
70 - 74 years	0.157%		
75 years	0.187%		
Stage specific diagnosis currently Stage 1: 2 : 3 : 4	18% : 11.5% : 27.5% : 43%		
Recurrence of Gastric Cancer in successfully treated patients by stage			
Recurrence in Stage 1 patients	11%	5% - 30%	Roukos <i>et al.</i> [6]
Recurrence in Stage 2 patients	53%	30% - 60%	
Recurrence in Stage 3 patients	83%	50% - 90%	

Utility Values (disutility*)			
Stage 1	0.88 (0.28)	0.78 – 0.98	Zhou HJ <i>et al.</i> [11]
Stage 2	0.86 (0.29)	0.72 – 0.99	
Stage 3	0.77 (0.31)	0.57 – 0.97	
Stage 4	0.68 (0.08)	0.52 – 0.84	
Test Characteristics			
Endoscopy Sensitivity	93%	-	Voutilainen <i>et al.</i> [12] Hamashima <i>et al.</i> [27]
Endoscopy Specificity	100%	-	Voutilainen <i>et al.</i> [12]
miRNA Sensitivity by Stages (Stage 1:2:3:4)	63% : 75% : 89% : 93%	30% - 100%	Current Study
miRNA specificity	89%	60% - 100%	Current Study

*Disutility refers to temporary reduction in QoL during first 6 months of treatment. Note: Assumed treatments are based on observed practice in Singapore. Gastric cancer patient on diagnosis undergoes staging investigation (CT, CXR, EUS & specialist consultation). Curative treatment includes surgery (total/ partial gastrectomy) & hospital stay (12days). Stage 3 patients undergo additional chemo-radiotherapy. Follow ups include: visits (2.2/year), repeat CT, CXR (1.4/year). Palliative care includes bypass surgery (30%), endoscopic stenting (6%), palliative chemotherapy (16%) & conservative treatment (2x specialist visits) (48%) with an appropriate hospital stay (12 days - on surgery, 2.5 days on average - if no surgery is performed). *Abbreviations used: CT: Computerized Tomography; CXR: Chest X-Ray; EUS: Endoscopic Ultrasound*

REFERENCE

- 1 Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, *et al.* Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;**3**:RESEARCH0034.
- 2 Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res* 2004;**64**:5245-50.
- 3 Singapore Cancer Registry Advisory Committee and Health Promotion Board S. Trends in Cancer Incidence in Singapore 2009-2013. 2014.
- 4 Singapore Cancer Registry. Cancer incidence and mortality 2003 - 2012 and selected trends 1973 - 2012 in Singapore. National Registry of Disease Office, 2015.
- 5 Department of Statistics MoTaI, Republic of Singapore. Population Trends 2016. 2016.
- 6 Roukos DH, Lorenz M, Karakostas K, Paraschou P, Batsis C, Kappas AM. Pathological serosa and node-based classification accurately predicts gastric cancer recurrence risk and outcome, and determines potential and limitation of a Japanese-style extensive surgery for Western patients: a prospective with quality control 10-year follow-up study. *Br J Cancer* 2001;**84**:1602-9.
- 7 Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005;**5**:13.
- 8 Dan YY, So JB, Yeoh KG. Endoscopic screening for gastric cancer. *Clin Gastroenterol Hepatol* 2006;**4**:709-16.
- 9 Zhou HJ, Dan YY, Naidoo N, Li SC, Yeoh KG. A cost-effectiveness analysis evaluating endoscopic surveillance for gastric cancer for populations with low to intermediate risk. *PLoS ONE* 2013;**8**:e83959.
- 10 Lee S, Jun JK, Suh M, Park B, Noh DK, Jung K-W, *et al.* Gastric Cancer Screening Uptake Trends in Korea: Results for the National Cancer Screening Program From 2002 to 2011: A Prospective Cross-Sectional Study. *Medicine* 2015;**94**:e533.
- 11 Zhou HJ, So JB, Yong WP, Luo N, Zhu F, Naidoo N, *et al.* Validation of the functional assessment of cancer therapy-gastric module for the Chinese population. Health and quality of life outcomes 2012;**10**:145.
- 12 Voutilainen ME, Juhola MT. Evaluation of the diagnostic accuracy of gastroscopy to detect gastric tumours: clinicopathological features and prognosis of patients with gastric cancer missed on endoscopy. *European journal of gastroenterology & hepatology* 2005;**17**:1345-9.
- 13 Li C, Li J, Cai Q, Qiu Q, Yan M, Liu B, *et al.* MiRNA-199a-3p: A potential circulating diagnostic biomarker for early gastric cancer. *Journal of surgical oncology* 2013;**108**:89-92.
- 14 Gorur A, Balci Fidanci S, Dogruer Unal N, Ayaz L, Akbayir S, Yildirim Yaroglu H, *et al.* Determination of plasma microRNA for early detection of gastric cancer. *Molecular biology reports* 2013;**40**:2091-6.
- 15 Cai H, Yuan Y, Hao Y-F, Guo T-K, Wei X, Zhang Y-M. Plasma microRNAs serve as novel potential biomarkers for early detection of gastric cancer. *Medical oncology (Northwood, London, England)* 2013;**30**:452.
- 16 Cui M-H, Hou X-L, Lei X-Y, Mu F-H, Yang G-B, Yue L, *et al.* Upregulation of microRNA 181c expression in gastric cancer tissues and plasma. *Asian Pacific journal of cancer prevention : APJCP* 2013;**14**:3063-6.
- 17 Li C, Li J, Cai Q, Qiu Q, Yan M, Liu B, *et al.* miRNA-199a-3p in plasma as a potential diagnostic biomarker for gastric cancer. *Annals of surgical oncology* 2013;**20 Suppl 3**:405.
- 18 Song M-y, Pan K-f, Su H-j, Zhang L, Ma J-l, Li J-y, *et al.* Identification of serum microRNAs as novel non-invasive biomarkers for early detection of gastric cancer. *PloS one* 2012;**7**.

- 19 Li B-s, Zhao Y-l, Guo G, Li W, Zhu E-d, Luo X, *et al.* Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection. *PloS one* 2012;**7**.
- 20 Valladares-Ayerbes M, Reboredo M, Medina-Villaamil V, Iglesias-Díaz P, Lorenzo-Patiño M, Haz M, *et al.* Circulating miR-200c as a diagnostic and prognostic biomarker for gastric cancer. *Journal of translational medicine* 2012;**10**:186.
- 21 Zhang W-H, Gui J-H, Wang C-Z, Chang Q, Xu S-P, Cai C-H, *et al.* The identification of miR-375 as a potential biomarker in distal gastric adenocarcinoma. *Oncology research* 2012;**20**:139-47.
- 22 Lo SS, Hung PS, Chen JH, Tu HF, Fang WL, Chen CY, *et al.* Overexpression of miR-370 and downregulation of its novel target TGFβ-RII contribute to the progression of gastric carcinoma. *Oncogene* 2012;**31**:226-37.
- 23 Tsujiura M, Ichikawa D, Komatsu S, Shiozaki A, Takeshita H, Kosuga T, *et al.* Circulating microRNAs in plasma of patients with gastric cancers. *Br J Cancer* 2010;**102**:1174-9.
- 24 Liu R, Zhang C, Hu Z, Li G, Wang C, Yang C, *et al.* A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. *European journal of cancer (Oxford, England : 1990)* 2011;**47**:784-91.
- 25 Liu H, Zhu L, Liu B, Yang L, Meng X, Zhang W, *et al.* Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer. *Cancer Lett* 2012;**316**:196-203.
- 26 Office NRoD. Cancer Incidence and Mortality 2003-12 and Selected Trends 1973-2012 In Singapore. Singapore Cancer Registry, Report No. 8. 2015.
- 27 Hamashima C, Okamoto M, Shabana M, Osaki Y, Kishimoto T. Sensitivity of endoscopic screening for gastric cancer by the incidence method. *International journal of cancer Journal international du cancer* 2013;**133**:653-9.