

Review Article

Diagnostic accuracy of Raman spectroscopy in malignant and benign colorectal lesions: a meta-analysis

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Abstract: Objective: Raman spectroscopy (RAS) is a novel non-invasive diagnostic method for colorectal cancer. This work aims to systematically analyze the rapid diagnosis of RAS in contrast with biopsy in patients with colorectal lesions. Methods: We searched a wide range of databases for all relevant data that assessed the diagnostic accuracy of RAS in detecting colorectal lesions with no language and time limitations. The pooled weighted estimates of sensitivity, specificity and related indicators were calculated by Meta-Disc Version 1.4 and STATA 12.0. The quality of included studies was assessed by the Quality Assessment of Diagnostic Accuracy Studies checklist 2. The Deeks' funnel plot asymmetry test was performed to evaluate publication bias. Results: The search strategy produced 113 hits after duplicates removal in which 14 articles were reviewed in this meta-analysis. A total of 1274 patients and 1660 lesions were assessed. Pooled weighted estimates of sensitivity and specificity of RAS in diagnosing colorectal cancer were 0.87 (95% CI, 0.86-0.89) and 0.89 (95% CI, 0.88-0.90), respectively. The positive likelihood ratio and the negative likelihood ratio were 6.72 (95% CI, 4.72-9.58), and 0.14 (95% CI, 0.09-0.20), respectively. The pooled diagnostic odds ratio and overall area under the curve of RAS in the diagnosis of colorectal cancer were 66.42 (95% CI, 32.90-134.08) and 0.9578. There was no significant publication bias ($P=0.34$). Conclusions: RAS has considerable sensitivity and specificity in the evaluation of colorectal lesions. RAS is a promising, reliable and non-invasive method for differential diagnosis of benign and malignant colorectal lesions.

Keywords: Raman spectroscopy, colorectal lesions, meta-analysis, diagnostic accuracy

Introduction

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females in the world, with approximately 1.4 million new cases and 693,900 deaths per year around the world [1]. Five-year survival rate is 90% if the disease is diagnosed while still localized to the wall of the bowel, but the rate is only 68% for regional disease with lymph node involvement, and only 10% if distant metastases are present [1]. Overall the incidence rates decrease by approximately 3% per year because of historical changes in risk factors (e.g. decreased smoking and red meat consumption, increased use of aspirin), the introduction and dissemination of early detection methods, and improvements in treatment for

colorectal cancer [2]. Among these, detecting disease early and having timely treatments are useful strategies to reduce colorectal cancer mortality rate [3]. Evidence and guidelines support several tests and strategies for colorectal cancer screening and early detection, including the stool tests, digital rectal examination, flexible sigmoidoscopy, colonoscopy, barium enema and computed tomographic colonography [4]. The stool tests and digital rectal examination are both convenient but non-specific for colorectal cancer diagnosis [5], and flexible sigmoidoscopy, barium enema and computed tomographic colonography for early identification of colorectal cancer have been found invasive, high-priced and possible to bring patients unexpected complications [6].

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In recent years, many studies have found that oncogenes in early cancer cells can encode characteristic proteins which have different structures and conformations from normal cells. Different types of lipids, proteins and nucleic acids have their own pattern of vibrations which can serve as Raman biomarkers [7]. Raman spectroscopy (RAS) can provide specific spectroscopic features and important biochemical macromolecules information including nucleic acids, proteins and lipids based on the inelastic light scattering. Therefore, RAS is a non-invasive, relatively specific, widely available and convenient tool, and a potential method for early diagnosis of diseases. RAS has been widely used in some tissues such as skin and lung [8], stomach [9], bladder and prostate [10] and breast [11] showing that it has reliable diagnostic value in various diseases. Colorectal cancer is also suitable to be detected by RAS because its micro structural variation is contrasted with normal colorectal tissue [12]. According to previous studies, the sensitivity of RAS in detecting colorectal cancer ranged from 71.4% to 100% and the specificity ranged from 74.1% to 100%. There are no systematic reviews or meta-analyses on this topic. The aim of this study is to systematically analyze the diagnostic performance of RAS on the detection of colorectal cancer with histopathology as the reference standard.

Material and methods

Literature search

Relevant studies were identified through a comprehensive search of PubMed, MEDLINE, EMBASE, Web of Science and Cochrane Library databases (up to November 1, 2015). The search terms were combinations of the relevant medical subject heading (MeSH) terms, key words, and word variants for “colorectal cancer” or “CRC” or “colorectal neoplasms” or “colorectal carcinoma” or “colon cancer” or “rectal cancer” and “Raman spectroscopy” or “Raman”. No language or time limitation was applied. We also performed a manual search to find other potential studies. Title, abstract and keywords for all related articles were scanned. Full-text papers were evaluated according to the following criteria to decide whether they were eligible. Two reviewers screened studies for eligibility independently. Disagreements were adjudicated by a third author.

Inclusion and exclusion criteria

Studies included in our research had to meet these criteria: (1) clinical studies conducted in humans focused on the diagnostic value of RAS for colorectal tissues, whether in vivo or ex vivo, (2) the gold standard for diagnosis was histopathology, (3) studies provided sufficient data to construct 2×2 contingency tables by extracting true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN), and (4) RAS was independently used or combined with other procedures to detect colorectal cancer.

Studies were excluded if they (1) were not focused on the diagnostic value of RAS for human colorectal cancer, (2) did not use histopathology as the gold standard, (3) lacked necessary data, (4) were duplicated publications, (5) were letters, reviews, case reports or editorial articles.

Data extraction

Data extraction included the first author's name, publication year of study, country, average age of patients, number of lesions, number of patients, reference standard, modalities, samples, in vivo/ex vivo, low frequency/high frequency and diagnostic algorithm. Raman spectra obtained from 0 to 2000 cm^{-1} were deemed to be of low frequency (LF), while spectra obtained from 2000 to 3800 cm^{-1} were of high frequency (HF) [13]. If one study had two diagnostic algorithms or it was conducted both in vivo and ex vivo or it had the accuracy of both low frequency and high frequency, we extracted all the different outcomes in these cases. TP, FP, TN, FN, positive predictive values (PPV) and negative predictive values (NPV) were collected directly or calculated according to the sensitivity and specificity in each reported study. Data extraction was performed independently by two reviewers. Divergences were assessed and resolved by a third reviewer.

Statistical analysis

All the statistical analyses were performed by STATA 12.0 and Meta-Disc Version 1.4. The diagnostic accuracy of RAS in detecting benign and malignant colorectal lesions was evaluated by calculating pooled sensitivity, specificity, negative likelihood ratio (NLR), positive likeli-

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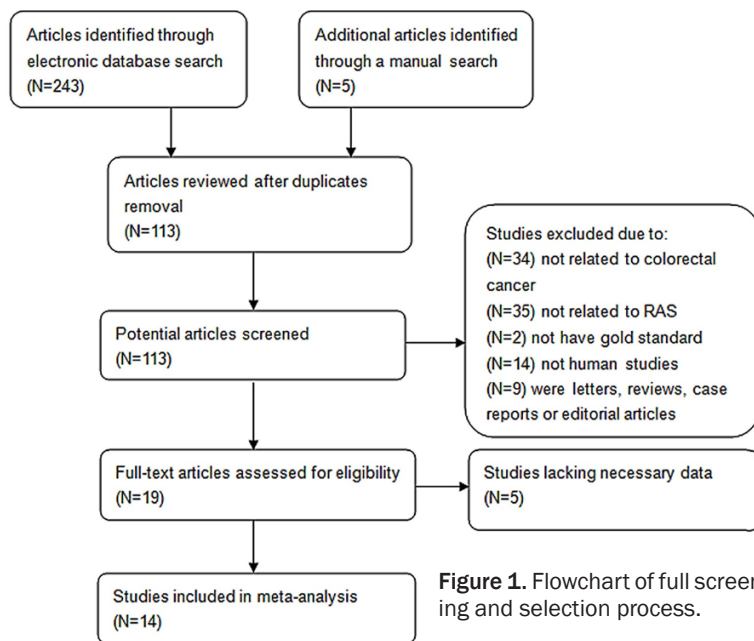


Figure 1. Flowchart of full screening and selection process.

hood ratio (PLR), overall area under the curve (AUC) and pooled diagnostic odds ratio (DOR) along with the corresponding 95% confidence intervals (95% CI) based on TP, FP, TN and FN from all studies. Sensitivity (proportion of test positives among people with disease) and specificity (proportion of test negatives among people without disease) were used to assess the diagnostic performance of RAS for detecting colorectal cancer. Epidemiological studies suggested that $PLR > 10$ had the value of making a definite diagnosis, while a low NLR (< 0.1) indicated a better diagnostic exclusion test [14]. In addition, a summary receiver operating characteristic (SROC) curve was constructed to investigate the impact of thresholds by the Moses-Shapiro-Littenberg method [15]. The AUC was calculated to show the diagnosis authenticity. The closer the AUC was to 1.0, the better the diagnosis authenticity was [16]. DOR is a conventional measurement of diagnostic performance including sensitivity and specificity and it is a reliable method to compare overall diagnostic accuracy among different tests [17]. The inconsistency index (I^2) and Chi-square test were used to detect the heterogeneity among studies. A P value < 0.05 suggested more heterogeneity existed than that expected by chance alone. An $I^2 > 50\%$ was considered significant for heterogeneity [18]. When statistical heterogeneity was identified, a random effect model was used, otherwise we selected fixed

effect model [19]. We conducted Deeks' funnel plot asymmetry test to investigate publication bias [20].

Quality assessment

Methodological quality of included studies was assessed independently by two reviewers using the Quality Assessment of Diagnostic Accuracy Studies checklist 2 (QUADAS 2). QUADAS 2 was constituted of four domains: (1) patient selection, (2) index test, (3) reference standard and (4) flow and timing [21]. The risk of bias and concerns about applicability were analyzed and evaluated as low risk, high risk and unclear risk

for each domain. QUADAS 2 was performed with Review Manager 5.2. Disagreements were resolved by a third reviewer.

Results

Study selection

The search strategy produced 113 hits after removing duplicates. Then we excluded 94 articles according to the exclusion and inclusion criteria by scanning the title, abstract and keywords of each record. Full texts and data integrity were also reviewed and 5 articles were further excluded because of lacking necessary data. Finally, 14 studies were included in this meta-analysis [13, 22-34]. The process of study selection was shown in **Figure 1**.

Study characteristics

The included studies were published from 2003 to 2015. The characteristics of the 14 studies were shown in **Table 1**. A total of 1274 patients and 1660 lesions were assessed. Some articles were analyzed more than once because they might include different diagnostic algorithms or frequencies, or were performed both in vivo and ex vivo. All these studies were published in English, and from different countries, including China ($n=6$), Singapore ($n=3$), Canada ($n=2$), Portugal ($n=1$), UK ($n=1$) and Czech Republic ($n=1$). Three studies were mea-

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Table 1. The characteristics of included studies

Study	Country	No. of patients	No. of lesions	No. of spectra	Samples	Algorithms	In vivo/ Ex vivo	Frequency	TP	FP	FN	TN
Molckovsky A, 2003	Canada	8	33	54	Tissue	PCA/LDA	Ex vivo	Low	31	1	3	19
Molckovsky A, 2003	Canada	8	33	54	Tissue	PCA/LDA	In vivo	Low	10	1	0	8
Chen K, 2006	China	8	16	320	Cells	PCA/LR	Ex vivo	Low	124	30	36	130
Zheng F, 2007	China	8	16	320	Cells	PCA/LR	Ex vivo	Low	124	30	36	130
Widjaja E, 2008	Singapore	59	105	508	Tissue	PCA/SVM	Ex vivo	Low	184	0	0	324
Lin D, 2011	China	83	83	83	Blood	PCA/LDA	Ex vivo	Low	37	0	1	45
Lopes PC, 2011	Portugal	48	48	48	Tissue	PCA/LDA	Ex vivo	Low	22	4	5	17
Chen Y, 2012	China	100	100	100	Blood	PCA/LDA	Ex vivo	Low	49	2	6	43
Li X, 2012	China	120	120	150	Blood	PCA/LDA	Ex vivo	Low	76	2	14	58
Li X, 2012	China	120	120	150	Blood	PLS/LDA	Ex vivo	Low	74	8	16	52
Ashok PC, 2013	UK	62	62	73	Tissue	PCA/SVM	Ex vivo	Low	39	7	5	22
Short MA, 2013	Canada	18	47	18	Tissue	PCA/LDA	Ex vivo	Low	9	1	0	8
Short MA, 2013	Canada	18	47	20	Tissue	PCA/LDA	Ex vivo	High	10	2	1	7
Wang J, 2014	China	206	206	206	Blood	PCA/LDA	Ex vivo	Low	103	0	0	103
Wang J, 2014	China	206	206	206	Blood	PLS/LDA	Ex vivo	Low	22	2	1	21
Bergholt MS, 2015	Singapore	49	121	302	Tissue	PLS/LDA	In vivo	Low	152	23	32	95
Bergholt MS, 2015	Singapore	49	121	302	Tissue	PLS/LDA	In vivo	High	10	1	1	5
Bergholt MS, 2015	Singapore	49	121	1228	Tissue	PLS/LDA	In vivo	High	93	132	6	997
Tatarkovic M, 2015	Czech Republic	55	55	55	Blood	PCA/LDA	Ex vivo	Low	20	7	8	20

PCA = principal component analysis, LDA = linear discriminant analysis, LR = logistic regression, PLS = partial least square, SVM = support vector machines; TP = true positive, FP = false positive, FN = false negative, TN = true negative.

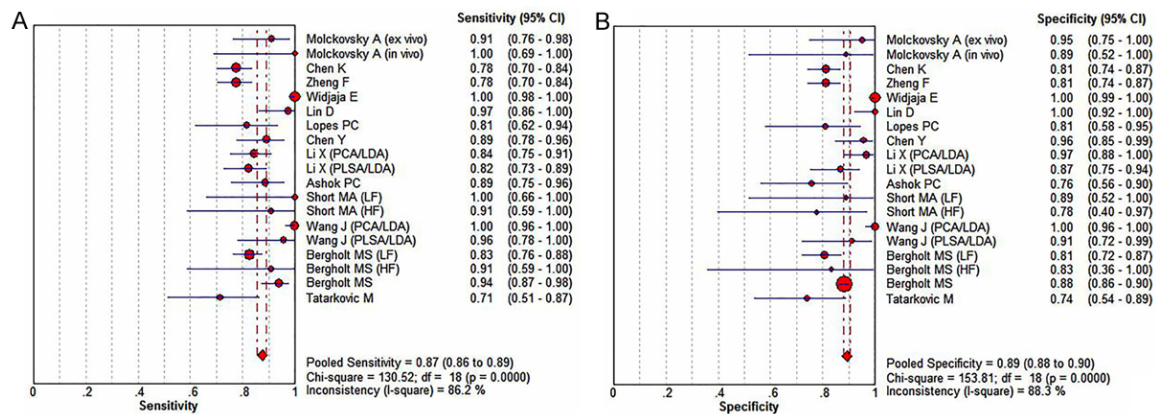


Figure 2. The pooled sensitivity and specificity for the diagnosis of colorectal cancer.

sured from in vivo tissues, while the others were from ex vivo tissues, single-cell suspensions or blood. Different diagnostic algorithms like principal component analysis (PCA), partial least square (PLS), or the combination with linear discriminant analysis (LDA) or support vector machines (SVM) were utilized in these eligible studies. In addition, eleven studies compared the diagnostic performance of low frequency RAS with histopathology, two studies compared both low and high frequency RAS

with histopathology, and one study presented the comparison of high frequency RAS and histopathology.

Overall analysis

The sensitivity, specificity, PLR, NLR, AUC and DOR were used to measure the diagnostic accuracy of RS in detecting colorectal cancer. The variation among studies was assessed by inconsistency index (I^2) and Chi-square test.

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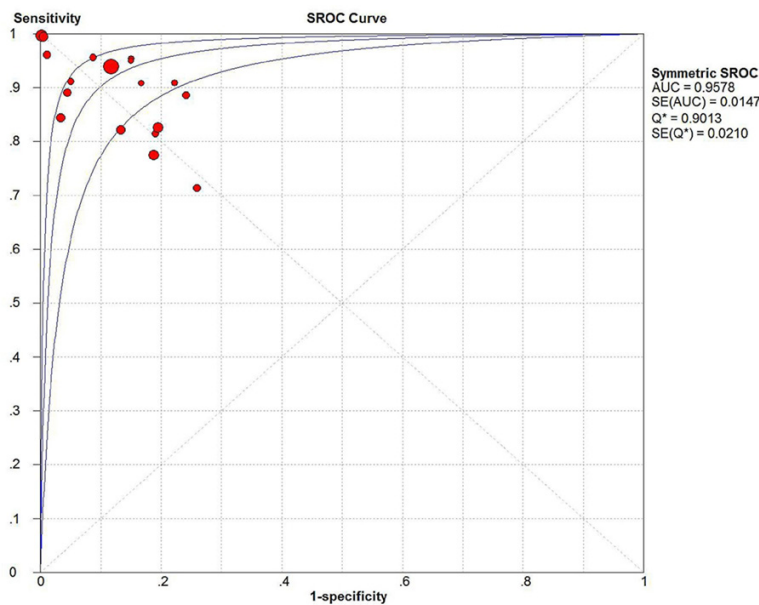


Figure 3. SROC curves with the overall area under the curve (AUC) of 0.9578.

The random effects model was used due to significant heterogeneity ($I^2 > 50\%$, $P < 0.05$) among the studies. The pooled sensitivity and specificity were 0.87 (95% CI, 0.86-0.89) and 0.89 (95% CI, 0.88-0.90), respectively. **Figure 2A, 2B** showed the forest plots of pooled sensitivity and specificity. The pooled PLR and NLR were 6.72 (95% CI, 4.72-9.58) and 0.14 (95% CI, 0.09-0.20), respectively. The pooled DOR was 66.42 (95% CI, 32.90-134.08). SROC curve was illustrated in **Figure 3** with the overall area under the curve of 0.9578 (standard error: 0.0147).

Subgroup analysis

To investigate the source of heterogeneity, we conducted subgroup analysis. When we grouped the studies by modalities, the pooled sensitivity, specificity and AUC of near infrared reflectance spectroscopy (NIRS) were 0.89 ($I^2=82.9\%$, $P=0$), 0.90 ($I^2=87.9\%$, $P=0$) and 0.9485, whereas the pooled data of Surface Enhanced Raman Scattering (SERS) were 0.96 ($I^2=77.4\%$, $P=0.0041$), 0.98 ($I^2=69.6\%$, $P=0.0196$) and 0.9942. When we grouped the studies by diagnostic algorithms, the pooled sensitivity, specificity and AUC of PCA were 0.88 ($I^2=89.0\%$, $P=0$), 0.91 ($I^2=90.6\%$, $P=0$) and 0.9648, while the pooled data for PLS were 0.86 ($I^2=65.5\%$, $P=0.0206$), 0.88 ($I^2=30.5\%$, $P=0.2183$) and 0.9146, respectively.

And when we grouped by in vivo or ex vivo studies, the pooled sensitivity, specificity and AUC of in vivo studies were 0.87 ($I^2=72.6\%$, $P=0.0121$), 0.88 ($I^2=44.4\%$, $P=0.1447$), and 0.8593, while the pooled data for ex vivo studies were 0.88 ($I^2=88.3\%$, $P=0$), 0.91 ($I^2=90.0\%$, $P=0$) and 0.9611, respectively. When we grouped by frequencies of Raman spectra, the pooled sensitivity, specificity and AUC of low frequency were 0.87 ($I^2=88.0\%$, $P=0$), 0.90 ($I^2=90.0\%$, $P=0$) and 0.9597, while the pooled data for high frequency were 0.93 ($I^2=0.0\%$, $P=0.8836$), 0.88 ($I^2=0.0\%$, $P=0.6353$) and 0.9946, respectively. The results of subgroup analysis in our meta-analysis were shown in **Table 2**.

Assessment of study quality and publication bias

QUADAS 2 was used to evaluate the methodological quality of each study. **Figure 4** presented the results of the evaluation of all those eligible studies. The risk of bias **Figure 4A** and concerns about applicability **Figure 4B** were evaluated as low risk. Overall, the quality of the studies was satisfactory.

In this work, Deeks' funnel plot asymmetry test was performed to evaluate publication bias in the included studies and the result was shown in **Figure 5**. There was no significant publication bias in this meta-analysis ($P=0.34$).

Discussion

Histopathology examination is the gold standard for colorectal cancer diagnosis. However, this diagnostic process is time-wasting, invasive, and it may bring patients anxiety and complications. To avoid unnecessary biopsies and diagnose cancer with satisfactory sensitivity and specificity, RAS is a reliable choice. RAS is a real-time, non-invasive optical method that can provide molecular changes between healthy and diseased tissues [35]. Chemical and structural changes in the molecular composi-

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Table 2. The results of subgroup analysis. Studies were grouped by country, diagnostic algorithms, in vivo/ex vivo and frequency of RAS

Subgroups	SEN (I ² , P-value, model)	SPE (I ² , P-value, model)	PLR (I ² , P-value, model)	NLR (I ² , P-value, model)	DOR (I ² , P-value, model)	AUC
All studies	0.87 (86.2%, P=0, REM)	0.89 (88.3%, P=0, REM)	6.72 (78.3%, P=0, REM)	0.14 (81.3%, P=0, REM)	66.42 (81.4%, P=0, REM)	0.9578
Country						
China	0.85 (87.6%, P=0, REM)	0.89 (88.1%, P=0, REM)	9.99 (82.0%, P=0, REM)	0.16 (78.7%, P=0, REM)	88.11 (84.7%, P=0, REM)	0.9668
Other countries	0.90 (84.2%, P=0, REM)	0.89 (89.4%, P=0, REM)	5.66 (77.3%, P=0, REM)	0.12 (83.5%, P=0, REM)	57.46 (79.6%, P=0, REM)	0.962
Modalities						
NIRS	0.89 (82.9%, P=0, REM)	0.90 (87.9%, P=0, REM)	6.21 (75.9%, P=0, REM)	0.14 (79.0%, P=0, REM)	55.62 (77.1%, P=0, REM)	0.9485
SERS	0.96 (77.4%, P=0.0041, REM)	0.98 (69.6%, P=0.0196, REM)	30.68 (53.3%, P=0.0929)	0.04 (69.0%, P=0.0216, REM)	865.04 (61.9%, P=0.0489, REM)	0.9942
Samples						
Tissue	0.91 (83.6%, P=0, REM)	0.90 (89.9%, P=0, REM)	6.29 (77.1%, P=0, REM)	0.10 (81.6%, P=0, REM)	76.14 (78.3%, P=0, REM)	0.9743
Cells*	-	-	-	-	-	-
Blood	0.89 (85.3%, P=0, REM)	0.94 (82.8%, P=0, REM)	14.9 (83.1%, P=0, REM)	0.12 (81.4%, P=0, REM)	159.57 (83.2%, P=0, REM)	0.9757
Algorithms						
PCA	0.88 (89.0%, P=0, REM)	0.91 (90.6%, P=0, REM)	8.79 (85.5%, P=0, REM)	0.13 (84.4%, P=0, REM)	87.96 (84.4%, P=0, REM)	0.9648
PLS	0.86 (65.5%, P=0.0206, REM)	0.88 (30.5%, P=0.2183, FEM)	6.34 (73.3%, P=0.0047, REM)	0.14 (72.4%, P=0.0059, REM)	48.83 (71.6%, P=0.0071, REM)	0.9146
In vivo/Ex vivo						
In vivo	0.87 (72.6%, P=0.0121, REM)	0.88 (44.4%, P=0.1447, FEM)	5.94 (81.5%, P=0.0010, REM)	0.11 (76.7%, P=0.0050, REM)	52.25 (75.5%, P=0.0066, REM)	0.8593
Ex vivo	0.88 (88.3%, P=0, REM)	0.91 (90.0%, P=0, REM)	8.51 (84.0%, P=0, REM)	0.14 (83.1%, P=0, REM)	78.67 (83.5%, P=0, REM)	0.9611
Frequency						
Low	0.87 (88.0%, P=0, REM)	0.90 (90.0%, P=0, REM)	7.69 (81.9%, P=0, REM)	0.15 (81.3%, P=0, REM)	66.53 (82.4%, P=0, REM)	0.9597
High	0.93 (0.0%, P=0.8836, FEM)	0.88 (0.0%, P=0.6353, FEM)	7.55 (0.0%, P=0.4501, FEM)	0.07 (0.0%, P=0.8013, FEM)	101.95 (0.0%, P=0.6128, FEM)	0.9946

SEN = sensitivity, SPE = specificity, PLR = positive likelihood ratios, NLR = negative likelihood ratios, DOR = Diagnostic odds ratio; AUC = overall area under the curve; REM = random effect model; FEM = fixed effect model; PCA = principal component analysis; PLS = partial least square. *Only 2 articles analyzed the diagnostic value of RAS based on cells, so the data were insufficient for meta-analysis.

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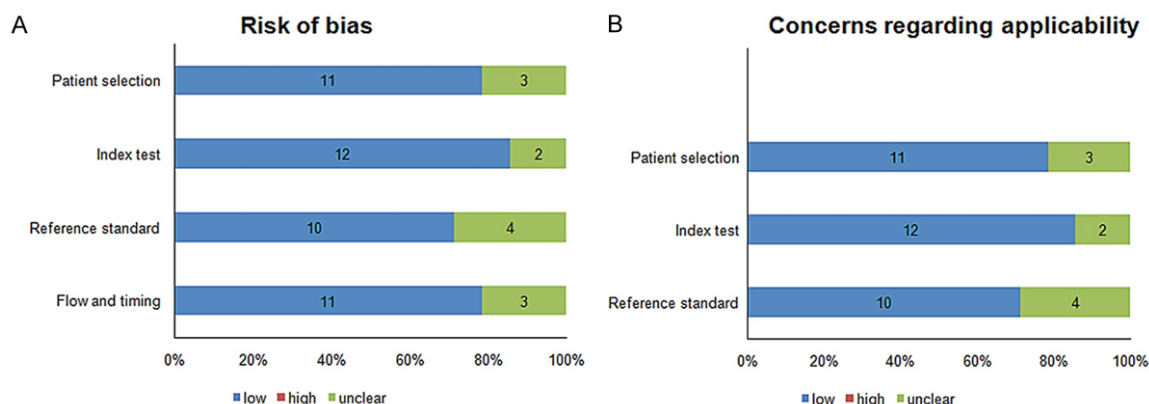


Figure 4. Quality assessment of included studies. The risk of bias (A) and concerns about applicability (B) were evaluated as low risk.

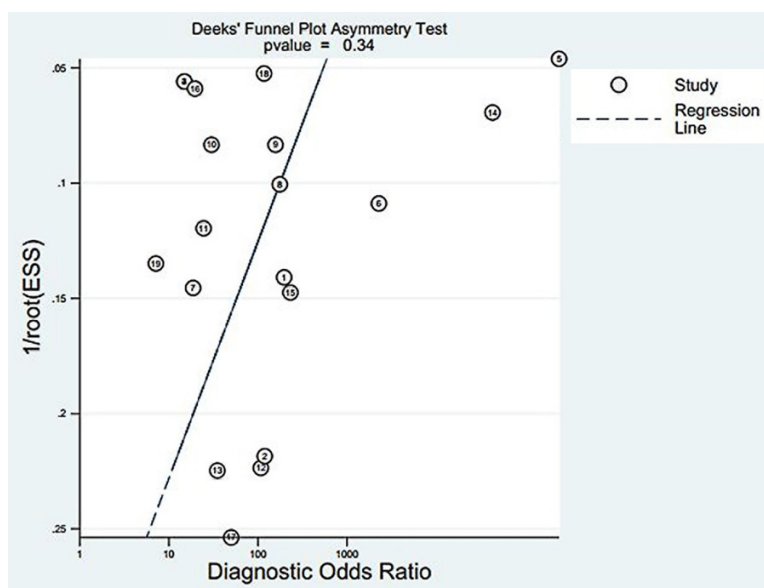


Figure 5. Deeks' funnel plot asymmetry test showed no significant publication bias ($P=0.34$).

tion have been found in the very beginning of cancer, which include higher metabolic activity, increased nucleus-to-cytoplasm ratio, changes in lipid and protein. All these changes may be exhibited by the absence or presence of Raman spectra peaks, and changes in peak intensities or peak shifts [36]. All of these are unique and specific for the early diagnosis of cancer. However, there is no evidence-based systematic review on the diagnostic accuracy of RAS in detecting colorectal cancer so far. Our meta-analysis with 14 included articles systematically evaluated the diagnostic performance of RAS on detecting colorectal cancer. We aimed

at assessing the value of RAS in clinical applications and providing theoretical foundation for clinicians. The overall sensitivity and specificity were 0.87 and 0.89, respectively. The pooled PLR and NLR were 6.72 and 0.14, respectively. The diagnostic accuracy quantified by AUC was 0.9578, and a diagnostic test is considered perfect if the AUC is 100%, excellent if greater than 90%, and good if greater than 80% [37]. All these results demonstrated that RAS had a considerable potentiality in differentiating colorectal cancers from normal colorectal tissues.

In recent years, rapid and early detection of colorectal cancer in vivo using non-invasive techniques has received more and more attention. Most clinicians desire to apply RAS which is a reliable, highly specific, optical, non-invasive technique to clinical decisions, which may improve prognosis of patients. In our research, we incorporated 15 ex vivo studies and 4 in vivo studies. The pooled sensitivity, specificity and DOR of in vivo and ex vivo studies were close, which suggested that RAS was a considerable tool and a promising application in differentiating colorectal cancer from normal tissues in vivo. More in vivo studies were urgent for demonstrating our outcome.

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Unlike the relatively featureless fluorescence-based techniques, Raman spectra are characterized by multiple sharp peaks and bands associated with various molecular vibration modes, which may better distinguish among different tissue types [22]. Nowadays, NIRS and SERS are common modalities of RAS currently under investigation. Conventional NIRS have high-sensitivity near-infrared detectors and filtered fiber-optic probes which make it possible to develop Raman spectroscopy instruments for clinical use [38]. Our present study has revealed the pooled sensitivity, specificity and DOR for NIRS are 0.89 ($I^2=82.9\%$, $P=0$), 0.90 ($I^2=87.9\%$, $P=0$) and 55.62 ($I^2=77.1\%$, $P=0$), suggesting that NIRS is a potential diagnostic method for colorectal lesions. With SERS technique, Raman signals can be enhanced by 13 to 15 orders of magnitude when the probed molecules are attached to nano-textured metallic surfaces, while the autofluorescence background can be greatly reduced at the same time [26]. Tumor detection based on SERS and gold nanoparticles under in vivo conditions has been conformed feasible [39]. Using RNA SERS or protein SERS in blood serum, or SERS with gold nanoparticle, the pooled sensitivity, specificity and DOR were 0.96 ($I^2=77.4\%$, $P=0.0041$), 0.98 ($I^2=69.6\%$, $P=0.0196$) and 865.04 ($I^2=61.9\%$, $P=0.0489$), respectively. The AUC of SERS was also higher than NIRS in our meta-analysis. Thus, SERS might be the better modalities for differential colorectal lesions at present.

According to previous studies, the traditional measurement range of low frequency (LF, 0 to 2000 cm^{-1}) has been demonstrated as a powerful analytical technique for differentiating lesions [40-42]. However, LF has several obstructions such as the difficulty to get good quality Raman spectra and the need to combine with other optical modalities, which make it challenging to be developed into a routine clinical tool [13]. An alternative is to use high frequency (HF, 2000 to 3800 cm^{-1}) range which has been tested to be slightly better at predicting the pathology than the LF range despite there being fewer Raman peaks in the HF measurement range [13]. In our work, we systematically analyzed the difference of diagnostic accuracy between LF and HF range. The pooled sensitivity, specificity and AUC of LF were 0.87, 0.90 and 0.9597, while the pooled data for HF were 0.93, 0.88 and 0.9946, respectively. The

NLR of HF was lower than 0.1. All these results suggested that HF might have potential capacity of detecting colorectal cancer.

Raman spectra reflect the overall biochemical changes of cancer cells through combination of two or more biomarkers which usually improves diagnostic results [43]. Most of these previous studies used linear mapping methods for data analyzing. For the early diagnosis of cancer utilizing Raman spectroscopy, PCA is the most common preprocessing method, which obtains an optimal number of principal components using heuristic or statistical approaches [44]. Another linear transform method most commonly used is PLS. It is an efficient dimension reduction method, which automatically selects loading vectors pertaining to the discriminate task [45]. Fourteen studies in our research used PCA and 5 used PLS. Most involved studies combine PCA or PLS with LDA or SVMs for classification analysis. LDA is a popular method for classification of colorectal cancer using RAS. For SVMs, the optimal generalization performance is achieved with high dimensionality data and/or dataset with low training samples to input dimensionality ratio [46]. If data has a normal distribution, LDA usually generalizes well when compared to SVMs [46]. The pooled sensitivity, specificity and AUC of PCA in this meta-analysis were 0.88, 0.91 and 0.9648, while the pooled data for PLS were 0.86, 0.88 and 0.9146, respectively. The pooled diagnostic accuracy of PCA was higher than PLS, which meant that PCA might perform better than PLS in feature extraction of spectral data.

We found significant heterogeneity among the 14 studies, so subgroup analysis was conducted to investigate potential sources of heterogeneity. In the subgroup analysis, heterogeneity still existed in certain subgroups with $I^2 > 50\%$. Potential sources could come from frequency, in vivo/ex vivo or feature extraction. For this reason, we used a random effect model if $I^2 > 50\%$, otherwise, we used fixed effect model. At the same time, we performed Deek's funnel plot to assess publication bias among the included studies. No publication bias was detected in this meta-analysis, thus publication bias was not a main source of heterogeneity.

Furthermore, our study has several limitations. First, because the pathological types and TNM staging of colorectal cancer were incomplete in

all studies, we could not sub-analysis pathological types and TNM staging. Second, the RAS analytical instruments might be different in these included articles, which might have introduced some bias to the analysis. Finally, RAS was widely used as an auxiliary diagnostic method for detecting colorectal cancer in non-western countries according to our retrieved results which might also resulted in some bias to the final analysis.

In this meta-analysis, we demonstrate that Raman spectroscopy has considerable sensitivity and specificity in the evaluation of colorectal lesions, which suggests that Raman spectroscopy is a promising, reliable method for differential diagnosis of benign and malignant colorectal lesions. We also suggest that SERS might be the better modalities for differential colorectal lesions; high frequency might have potential capacity in detecting colorectal cancer and PCA may perform better than PLS in feature extraction of spectral data. RAS is a considerable tool and a promising application in differentiating colorectal cancer from normal tissues *in vivo*. Further studies are still required to confirm our findings.

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Disclosure of conflict of interest

None.

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