Point-of-Care Diagnosis of Bladder Cancer With Vibrational Spectroscopy: A Systematic Review

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Abstract

Introduction Vibrational spectroscopy (VS) is a new and rapidly evolving technology in cancer diagnostics. Originating from analytical chemistry, VS evaluates vibrations of nuclei to produce a unique "biological fingerprint." While multiple studies have been published on this technology and physician awareness has increased, no systematic review has evaluated the role of VS in bladder cancer (BCa) tissue diagnosis.

Methods To conduct this systematic review, we searched the MEDLINE, Embase, and Cochrane databases for studies that used Raman spectroscopy (RS), surface-enhanced RS (SERS), infrared spectroscopy (IR) or near-infrared spectroscopy (NIRS) to analyze human BCa specimens. Studies using animal tissue or liquid biopsies were excluded. We synthesized the evidence by comparing modalities, study design, data analysis techniques, and diagnostic accuracy. The quality of evidence was evaluated by the QUADAS-2 tool.

Results Out of 362 results, 20 studies met our inclusion criteria. There has been growing interest in VS use in BCa, with 50% of the studies published in the past 5 years. RS was the most commonly used modality (65%), followed by IR (20%) and SERS (10%). Only one study compared RS to IR (5%). The mean sample size was 44 patients (range, 6–214). To date, there have been only 2 in vivo studies, with the remaining ex vivo studies performed with large variation in tissue preparation, data analysis, and reporting. Advancements in fiber optic probes and machine-learning data analysis techniques, and increased computational power have improved diagnostic accuracy up to 98% sensitivity and 100% specificity.

Conclusions VS shows high potential for BCa diagnosis, but there is a need for uniform reporting methods and studies with adequate sample sizes to validate the models. RS has shown promising results, with ongoing improvements in fiber optic probes allowing its integration into conventional cystoscopes. While no single VS modality has proven to be perfect, a multimodal approach is likely required to establish its value in clinical practice.

Introduction

Challenges in bladder cancer diagnosis

Bladder cancer (BCa) is among the 10 most common cancer types worldwide, with approximately 550 000 new cases annually[1]. The recurrence and progression rates vary greatly, based on factors such as tumour grading, size, depth of invasion, and presence of carcinoma in situ (CIS). At 5 years, the recurrence rates range from 31% to 78% and the progression rates range from 1% to 45%[2]. The stage of cancer is the most important prognostic factor, highlighting

Key Words

Urinary bladder neoplasms, diagnosis, spectroscopy, Raman spectroscopy, infrared spectroscopy

Competing Interests

None declared.

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Abbreviations

ANN artificial neural network
AUC area under the curve
BCa bladder cancer
CIS carcinoma in situ
FFPE formalin-fixed paraffin-embedded
FT-IR Fourier transform infrared
IR infrared
NIRS near-infrared spectroscopy
PCA principal component analysis
QCL quantum-cascade laser
QUADAS-2 Quality Assessment of Diagnostic Accuracy Studies
RS Raman spectroscopy
SERS surface-enhanced Raman scattering
TURBT transurethral resection of bladder tumour
VS vibrational spectroscopy

the importance of techniques for accurately and efficiently diagnosing BCa stage in controlling disease progression.

The current gold standard diagnostic method is white-light cystoscopy, followed by biopsies or transurethral resection of bladder tumour (TURBT) for histopathological examination. While white-light cystoscopy is reliable for papillary tumours, it has limitations in detecting flat carcinomas such as CIS, dysplasia, and multifocal lesions. Integrated findings from 2 fluorescence cystoscopy registration studies revealed that only 38% of CIS lesions[3] and 71% of CIS cases were detected using white-light cystoscopy[3,4]. Although newer optical techniques such as fluorescence and narrow-band imaging can improve tumour visualization, they do not contribute to histopathologic diagnosis[5]. Thus, repeated biopsies are often performed, a procedure that not only is costly but also does not provide real-time point-of-care diagnosis. Delays in diagnosis and treatment may lead to increased morbidity and mortality, particularly for high-risk invasive BCa. A more rapid and cost-effective diagnostic method would potentially enhance management of BCa patients.

Vibrational spectroscopy

Spectroscopy has attracted attention in cancer diagnosis in recent years. Originating from analytical chemistry, vibrational spectroscopy (VS) is a powerful technique that measures the vibrational energy of molecules[6]. The 3 most common techniques used in cancer detection are infrared (IR), Raman (RS), and near-infrared (NIR) spectroscopy. The key characteristics, advantages, and limitations of each technique are summarized in **Table 1**[7].

IR absorption spectroscopy relies on the absorption of mid-IR radiation by the sample, where molecules absorb specific frequencies of light based on their unique structure. This allows for identification and quantification of the molecular compound in a sample. The exact frequency required to excite a molecular vibration depends on the mass of the atoms involved in the vibration and the type of chemical bonds between these atoms, which can be influenced by a molecule's structure and chemical microenvironment[8].

RS is a complementary method based on inelastic light scattering. In this method, the sample is illuminated with monochromatic laser light, and the interactions between molecules and photons leads to the scattering of light. The energy of the scattered light reflects the molecular composition of the sample[8]. RS offers several advantages over IR spectroscopy, including less interference from water and glass, less sample preparation, and higher spatial resolution. However, RS is inherently weaker and requires longer spectral scanning times to achieve an adequate signal-to-noise ratio. Another significant limitation of RS is the presence of strong fluorescent signals, particularly in the analysis of organic tissues, dramatically reducing its specificity and hampering its clinical translation.

Surface-enhanced Raman scattering (SERS) spectroscopy is the newest technique that aims to overcome the limitations of conventional RS. SERS uses plasmonic substrates, such as silver or gold colloids, to amplify the Raman signal of molecules adsorbed onto the metal surface[9]. This technique holds great potential in identifying BCa in liquid biopsies such as urine or serum, but its use in tissue is still emerging[10].

Compared with its counterparts, mid-IR and RS, near-infrared (NIR) spectroscopy has received less historical research attention. Contrary to the mid-IR region, which relies on distinct fundamental absorption bands, the NIR region contains overlapping overtone and combination bands that have lower intensity and reduced specificity[11]. However, recent advancements in quantum mechanical calculations and computational power have greatly expanded the use of NIR in modern analytical applications[12]. NIR offers advantages such as easier sample handling, low cost, greater sample penetration, and rapid acquisition times.

All 3 techniques—IR, RS, and NIRS—can analyze biological tissues, which comprise the superposition of biochemical components such as DNA, proteins, lipids, and carbohydrates. VS can capture the unique "biological fingerprint" of the entire sample under analysis, rather than focusing on single elements like cell morphology in histopathology or tumour DNA in assays. VS has the potential to evaluate the entire phenotypic response of the host, including tissue changes

TABLE 1.
Overview and comparison of the three common vibrational spectroscopy methods used in tissue analysis

		Mid-IR	NIR	Raman
Basis	Method of detection	Mid-infrared light absorption using polychromic light source	Near-infrared light absorption using polychromic light source	Inelastic light scattering using monochromic laser excitation
	Wavenumber	400–4000 cm-1	4000–10 000 cm-1	50–4000 cm-1
	Wavelength	2500–25 000 nm	1000–2500 nm	2500–20 0000 nm
Sampling Methods	Sample interface	Historically complicated	Straightforward point-and-shoot, versatile	Straightforward point-and-shoot
	Sample preparation	Complex	Minimal	Minimal
	Penetration of glass / quartz / plastic	No	Yes	Yes
	Probes	Probes fragile	Fiber optic probe compatible	Fiber optic probe compatible
Sample Size	Area	1–2 mm	Up to several cm	0.3–1 mm
	Depth	< 15 µm	< 1 mm to several mm	Surface spectra from bulk material possible
Sensitivity	Water	High	Medium	Low
	Coloured samples	No issue	No issue	Susceptible to fluorescence
Specificity	Chemical	High	Medium	High
Observed Bands		Fundamentals—narrow	Combinations and overtones—overlapping	Fundamentals—narrow

IR: Infrared; NIR: Near-infrared

such as protein/lipid ratio, tumour characteristics, and immune cell interactions, encompassing a multimarker approach to cancer diagnostics[13]. In recent years, proof-of-concept studies in breast, colon, skin, and bladder cancers have demonstrated that VS can be employed as a label-free, non-destructive, and non-invasive approach to specimen analysis, facilitating the identification of specific "spectral biomarkers"[14].

Objective of this review

Despite the increasing literature and public awareness into the role of VS in BCa tissue diagnosis, no systematic review has covered the topic. This review aims to

- Provide a historical overview of the development of VS in BCa diagnosis.
- Compare the 3 most common VS techniques—IR, RS, and NIRS.
- Assess the feasibility and diagnostic accuracy of studies.
- Identify future areas of research based on the current literature.

Methods

This review was performed in accordance with the PRISMA 2020 statement[15]. It was registered with the International Prospective Register of Systematic Reviews (PROSPERO #CRD42022349369), where the protocol and search strategy are available.

Eligibility criteria

A summary of eligibility criteria for this review, following the PICO framework (Population, Intervention, Comparison, Outcome) is detailed in **Table 2**. Studies of humans with either ex vivo or in vivo vibrational spectral analysis of bladder tissue for the detection of cancer were included. There were no demographic restrictions. Tissue samples required analysis by VS in a laboratory or operating theatre for inclusion. Types of VS considered included RS, NIRS, and IR spectroscopy. Histopathology was required as the reference standard. Publications reporting any diagnostic capability of VS were included. There were no restrictions on language or publication date. Studies involving animal tissue, pooled cells, tissue markers, or liquid biopsies were excluded. Only peer-reviewed

TABLE 2.

Criteria for studies included in this systematic review

Inclusion criteria	
Population	Human bladder cancer tissue
Investigation	Vibrational spectroscopy modalities: 1. Raman (RS) 2. Fourier transform infrared (FT-IR) 3. Near-infrared (NIRS)
Control	Histopathology
Outcomes	Quantitative: Diagnostic accuracy, sample size, scan time, and excitation laser wavelength Qualitative: Study design and limitations, tissue preparation, algorithm for analysis, and the pathological groups compared
Setting	Intraoperative, bedside, or laboratory

articles were considered, with review articles, opinion papers, and commentaries excluded.

Search strategy

An online electronic database search was undertaken using the platforms of MEDLINE, Embase, and Cochrane Library. The search encompassed the entire database content. The initial search employed broad MeSH terms including "Urinary Bladder Neoplasms/" and (Spectrum analysis, Raman OR spectroscopy, near-infrared/ OR Spectrophotometry, Infrared/)while also extracting key terminology/key words from reviews and a sample of potentially relevant primary data studies. A gold test set of relevant studies was used to ensure the search terms retrieved all of the gold test set. The results of the literature search were downloaded into EndNote X9 software (Clarivate Analytics, London, UK) and exact article duplicates were removed using the duplicate tool in that software program. Subsequently, a reference review of identified articles and reviews was conducted to identify any additional relevant articles. Grey literature was searched via guidelines from the European Association of Urology (EAU), American Urological Association (AUA), and National Institute for Health and Care Excellence (NICE) and ongoing clinical trials through ClinicalTrials.gov, The ISRCTN registry, and the World Health Organization International Clinical Trials Registry Platform (ICTRP) portal. The authors of trials were contacted for preliminary or unpublished results for potential inclusion in the review. Full search strategy and results are provided in **Online Appendix 1**.

Selection process

Following completion of the search, all identified citations were uploaded into Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia) and duplicates were removed. The screening for inclusion was conducted in 2 phases. The first phase involved screening titles and abstracts from the initial search results. The second phase involved reviewing full-text articles based on the previously stated inclusion criteria. Both phases of screening were conducted by 2 independent reviewers (A.Y. and M.A.). In cases of unresolved disagreements, a third senior reviewer (D.B.) acted as an adjudicator. The same approach was used to screen all grey literature sources.

Data collection process

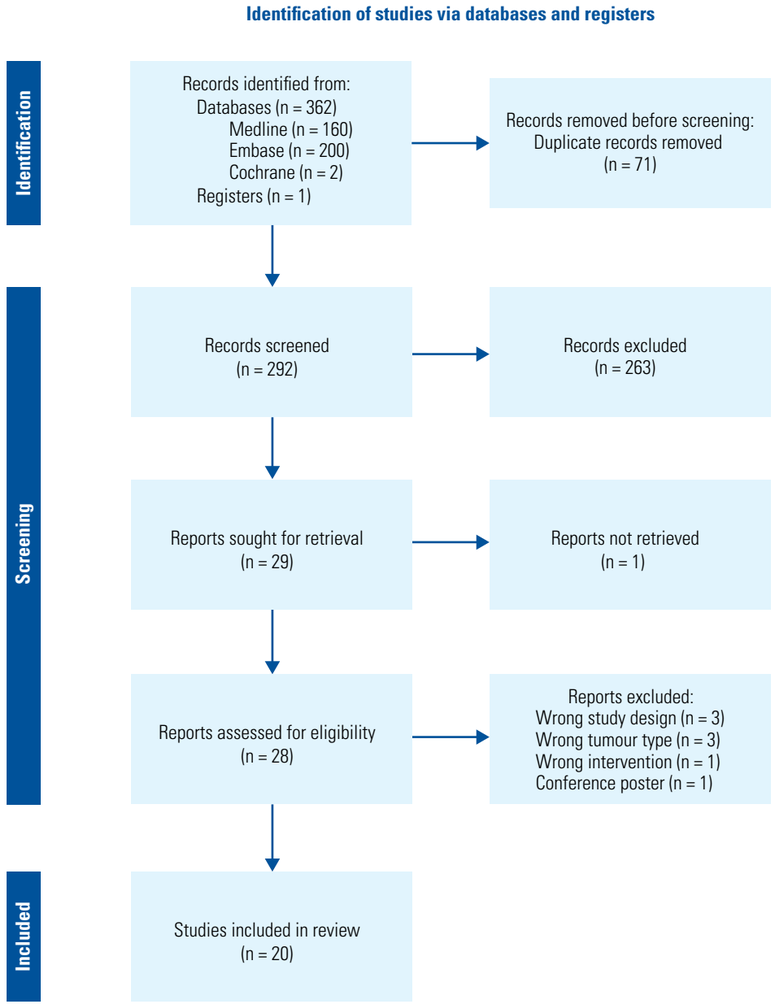
Two reviewers (A.Y. and M.A.) independently conducted data extraction onto a predefined extraction sheet. The extracted data were cross-checked independently. The primary outcome measures extracted for assessing the effectiveness of a diagnostic modality included quantitative measures of accuracy such as sensitivity, specificity, overall accuracy, and area under the curve (AUC) values. Secondary outcome measures encompassed both quantitative and qualitative data covering study design and limitations, tissue preparation, scan time, excitation laser wavelength, data analysis technique, and comparison of pathological groups. If multiple data analysis techniques were evaluated within a study, the data described are based on the most effective technique used. When data were presented for both a training set and test/cross-validation set, the data from the test set are presented, as it reflects the performance of the test in clinical practice most closely.

Study risk of bias assessment

Two reviewers independently assessed each eligible study using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool[16]. Any areas of conflict between the 2 reviewers were resolved through arbitration involving a third reviewer (D.B.), if necessary.

FIGURE 1.

PRISMA 2020 diagram of study selection



Results

A total of 363 articles were identified through literature search, of which 263 were excluded on screening. Of 29 full-text articles assessed for eligibility, 20 were included in this review (**Figure 1**).

Characteristics of the included studies

Table 3 provides a summary of the characteristics of the included studies, while **Table 4** contains a summary of all data collected. There has been a growing interest in spectroscopy and BCa since the publication of the first study in 2004, with 10 of 20 studies published in the past 5 years. The most commonly used modality was RS (65%), followed by IR (20%), and SERS (10%). No studies using NIRS were found. Two comparator studies were included. One compared FT-IR and RS on the same bladder specimens[17], while another compared a novel superficial RS fiber optic probe with a non-superficial probe[18].

Sample sizes of the studies were low, with a mean of 44 patients (range, 6–214). Only 2 studies were conducted in vivo[18,19], while the remaining studies were ex vivo (n = 18). There was considerable variation in tissue preparation methods, including snap-freezing bladder specimens post-TURBT and subsequently thawing (40%),

TABLE 3.

Characteristics of studies included in the systematic review

Characteristic		Studies n (%)
Period of publication	2004–2009	5 (25)
	2010–2016	7 (35)
	2017–2022	10 (50)
Spectroscopy modality	Raman	14 (65)
	FT-IR	5 (20)
	SERS	2 (10)
	Raman & FT-IR	1 (5)
	NIRS	0 (0)
Sample size	< 20	6 (30)
	20–50	10 (50)
	50	4 (20)
Tissue preparation	In vivo	2 (10)
	Ex vivo: frozen	8 (40)
	Ex vivo: fresh	6 (30)
	Ex vivo: formalin	6 (30)
Data analysis technique	PCA-LDA	9 (45)
	PCA-SVM	2 (10)
	PLS-LDA	2 (10)
	PCA	2 (10)
	OLS	1 (5)
	PCA-ANN	1 (5)
	Constituents only	5 (25)
Histopathological categories compared	Benign vs. cancer	18 (90)
	Grade characterization	6 (30)
	Stage characterization	3 (15)
	Subtype characterization	2 (10)

ANN: artificial neural networks; CAS: cluster-averaged spectra; FT-IR: Fourier transform infrared; LDA: linear discriminant analysis; NIRS: near-infrared spectroscopy; OLS: ordinary least squares regression; PCA: principal component analysis; PLS: partial least squares; SERS: surface-enhanced Raman spectroscopy; SVM: support vector machines.

immediate analysis of fresh specimens post-TURBT (30%), or placing specimens into formalin that was variably reversed before spectral scanning (30%).

Regarding data analysis techniques, principal component fed linear discriminant analysis (PCA-LDA) was the most commonly used technique (45%). Other analysis methods included support vector machines, partial least squares linear discriminant analysis, cluster-averaged spectra, ordinary least squares regression, principal component analysis, and artificial neural networks. The quantitative measures of accuracy varied greatly across studies. While not all studies reported sensitivity and specificity, some chose to report overall accuracy and AUC. Additionally, 25% of the studies provided only descriptive analysis of tissue constituents such as proteins, lipids, DNA, collagen, and cholesterol.

Comparing the performance of spectroscopic techniques

Quantitative measures of diagnostic accuracy, such as sensitivity and specificity, were reported in 12 of 14 studies that used RS. The remaining 2 studies reported descriptive analysis of tissue constituents instead of accuracy[17,20]. Diagnostic endpoints varied significantly across studies, depending on the histopathological categories chosen for analysis. For example, 90% of the studies compared benign to malignant tissues, while 30% compared low-grade with high-grade urothelial cancer. This variability made direct comparisons between studies difficult. Overall, the sensitivity and specificity for detecting malignancy ranged from 71% to 97% and from 72% to 100%, respectively.

In contrast, only 1 of 5 FT-IR studies reported on accuracy. Hughes et al. (2013) used support vector machines to achieve a class accuracy of 98% to 99% when distinguishing conventional urothelial cancer from rare subvariants. They did not compare with benign tissues[21]. Pezzei et al. (2013) used FT-IR microscopic imaging with tissue microarray technology to correlate with stained histological BCa tissue sections, opening up new possibilities for spectroscopic analyses and exploration of the molecular changes associated with histopathological morphology[22]. The remaining 3 FT-IR studies reported only on concentrations of tissue constituents.

Two studies with markedly different study designs used SERS. The first study, conducted by Jin et al. (2019), compared luminal and basal-like subtypes of BCa and reported an overall accuracy of 94%[23]. That study used 50 snap-frozen specimens without any benign controls. In a subsequent study, Zacharovas et al. (2022) applied SERS to freshly excised bladder tissue and extracellular fluid. Their 3-group algorithm achieved a sensitivity of

FIGURE 2.

Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) analysis of the included studies

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Crow et al., 2004	Low	Low	Low	Low	Low	Low	Low
Crow et al., 2005	High	High	Low	High	Low	Low	Low
de Jong et al., 2006	High	Low	Low	Unclear	High	High	Low
Stone et al., 2007	High	Low	Low	Unclear	Low	High	Low
Draga et al., 2010	Low	High	High	High	Low	Low	Low
Ahmed et al., 2010	Unclear	High	Low	Unclear	High	High	Low
Barman et al., 2012	Low	Low	Low	Low	Low	Low	Low
Al-Musletet al., 2012	Unclear	Low	Low	Unclear	High	High	Low
Pezzeiet al., 2013	High	High	Low	High	High	High	Low
Hugheset al., 2013	High	High	Low	Unclear	High	High	Low
Chenet al., 2018	Low	Low	Low	Low	Low	Low	Low
Jinet al., 2019	High	High	Unclear	Unclear	High	High	Unclear
Pavlovet al., 2019	Low	Low	Unclear	Low	Low	Low	Unclear
Yousifet al., 2020	High	High	Low	Low	Low	High	Low
Placzeket al., 2020	Low	Low	High	High	Low	Low	Low
Corderoet al., 2020	Low	Low	High	High	Low	Low	Low
Morselliet al., 2021	Low	Low	Low	High	Low	High	Low
Zacharovas et al. (2022)[10]	Low	High	Low	High	Low	High	Low
Stomp-Agenantet al., 2022	High	High	Low	High	High	Low	Low
Taieb et al., 2022	High	High	Low	High	High	High	Low

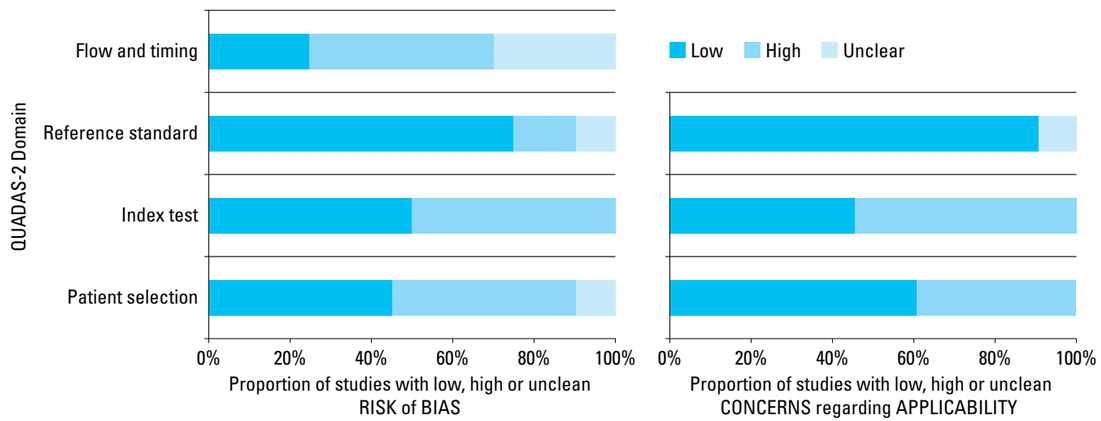


TABLE 4.

Summary of all data collected for the included studies

Study/Year	Country	Patients/ Specimens	Malignant/ Control	Tissue preparation	Spectroscopy modality/ Mean scan time		Laser wavelength	Data analysis technique	Histopathological categories compared	Diagnostic endpoint	
										Sensitivity / Specificity	Accuracy / Other
Crow et al. (2004)[24]	United Kingdom	72/75	3/22	Ex vivo—Snap-frozen post-TURBT	Raman / 10 sec		830 nm	PCA-LDA	• (i) normal, (ii) cystitis, (iii) malignant • (i) low grade, (ii) high grade • (i) pTa, (ii) pT1, (iii) pT2	91% / 98% 93% / 98% 96% / 96%	n/a
Crow et al. (2005)[40]	United Kingdom	24/29	10/19	Ex vivo—Snap-frozen post-TURBT	Raman, fibreoptic probe / 5–30 sec		785 nm	PCA-LDA	• (i) benign, (ii) malignant	79% / 89%	Accuracy 84%
de Jong et al. (2006)[41]	Netherlands	15/15	9/6	Ex vivo –Snap-frozen post-TURBT	Raman map / 20 sec		845 nm	PCA-LDA CAS	• (i) benign, (ii) malignant	94% / 92%	Accuracy 98%
Stone et al. (2007)[20]	United Kingdom	24/73	41/32	Ex vivo—Snap-frozen post-TURBT	Raman / 20 sec		830 nm	OLS	• (i) benign, (ii) malignant	n/a	Constituents: actin, collagen, choline, triolein, DNA, cholesterol
Draga et al. (2010)[19]	The Netherlands	38/63	23/29	In vivo—Live tissue prior to TURBT	Raman and PDD / 1–5 sec		785 nm	PCA-LDA	• (i) normal, (ii) cystitis, (iii) malignant • (i) normal, (ii) cancer • (i) normal, (ii) pTa, (iii) pT1 + pT2	71% / 87% 85% / 79% 58% / 76%	n/a
Ahmed et al. (2010)[17]	Sudan	7/14	4/0	Ex vivo—formalin, dried, grinded, KBr additive	Raman and FT-IR / Scan time n/a		1064 nm	Constituents only	• (i) benign, (ii) malignant	n/a	Constituents: proteins, lipids, nucleic acids
Barman et al. (2012)[25]	United Kingdom	14/28	14/14	Ex vivo—fresh post-TURBT	Raman,confocal probe / 5 sec		785 nm	PCA-LDA	• (i) benign, (ii) malignant	86% / 100%	Accuracy 93% AUC 0.91
Al-Muslet et al. (2012)[42]	Sudan	11/22	11/11	Ex vivo—formalin, dried, grinded, KBr additive	FT-IR / Scan time n/a		n/a	Constituents only	• (i) benign, (ii) malignant	n/a	Constituents: proteins, lipids, nucleic acids
Pezzei et al. (2013)[22]	Austria	214	214/0	Ex vivo—formalin, dried	FT-IR micro-spectroscopy / Scan time n/a		n/a	PCA	• (i) benign, (ii) malignant	n/a	n/a
Hughes et al. (2013)[21]	United Kingdom	6/6	6/0	Ex vivo—reversal of FFPE tissue	FT-IR micro-spectroscopy / Scan time n/a		n/a	PCA-SVM	• Rare subvariants only: (i) conventional urothelial cancer, (ii) micro-papillary, (iii) stroma, (iv) lymphocyte-rich, (v) clear cell, (vi) lipoid	n/a	Accuracy 98%
Chen et al. (2018)[26]	China	10/32	21/11	Ex vivo—snap-frozen in liquid nitrogen	Raman, fiber optic probe / 1 sec		785 nm	PCA-ANN	• (i) normal, (ii) low grade, (iii) high grade	90% / 98% LG 98% / 96% HG	Accuracy 93%
Jin et al. (2019)[23]	China	50	50/0	Ex vivo—snap-frozen	SERS / 10 sec		633 nm	PCA-LDA	Two subtypes: • (i) luminal, (ii) basal-like	n/a	Accuracy 94% AUC 97
Pavlov et al. (2016)[43]	Russia	22	22/13	Ex vivo—fresh post-TURBT	Raman / Scan time n/a		785 nm	PCA-LDA	• (i) benign, (ii) malignant	97% / 96%	n/a
Yousif et al. (2020)[44]	Iraq	46/46	23/23	Ex vivo—formalin	ATR-FT-IR / Scan time n/a		n/a	Constituents only	• (i) benign, (ii) malignant	n/a	Constituents: proteins, lipids, collagen
Placzek et al. (2020)[27]	Denmark	44/119	53/66	Ex vivo—fresh or snap-frozen	Raman and OCT / Scan time n/a		785 nm	PLS-LDA	• (i) benign, (ii) malignant • (i) low grade, (ii) high grade	95% / 88% 81% / 61%	n/a
Cordero et al. (2020)[35]	Denmark	28/67	37/11	Ex vivo—fresh or snap-frozen	Raman, fiber optic mapping / 3 sec		785 nm	PLS-LDA	• (i) benign, (ii) malignant • (i) low grade, (ii) high grade	92% / 93% 85% / 83%	Accuracy 92% Accuracy 84%
Morselli et al. (2021)[28]	Italy	114/169	40/129	Ex vivo—fresh post-TURBT	Raman, fiber optic & fluorescence & reflectance / Scan time n/a		785 nm	PCA-LDA	• (i) benign, (ii) malignant • (i) low grade, (ii) high grade • (i) pTa, (ii) pT1 • (i) pTa, (ii) pT2 • (i) pT1, (ii) pT2	77% / 72% 73% / 65% 65% / 71% 81% / 81% 75% / 76%	Accuracy 77%
Zacharovas et al. (2022)[10]	Lithuania	30/58	25/28	Ex vivo—fresh post-TURBT	SERS /5 min		1064 nm	PCA	• (i) normal, (ii) cystitis, (iii) malignant	85% / 97%	n/a
Stomp-Agenant et al. (2022)[18]	The Netherlands	75/117	51/66	In vivo—Live tissue prior to TURBT	Raman fiber optic, superficial vs normal probe / 0.5 sec		785 nm	PCA-LDA	• (i) benign, (ii) malignant • (i) normal, (ii) low grade, (iii) high grade	90% / 87% superficial probe 80% / 85% normal probe	AUC 0.95 superficial probe AUC 0.80 normal probe
Taieb et al. (2022)[45]	Israel	Unknown	Unknown	Ex vivo—reversal of FFPE tissue	Raman mapping / 60 sec		561 nm	PCA-SVM	• (i) benign, (ii) malignant	84% / 88%	n/a

ANN: artificial neural networks; ATR: attenuated total reflection; AUC: area under the curve; CAS: cluster-averaged spectra; FFPE: formalin-fixed paraffin-embedded; FT-IR: Fourier transform infrared; HG: high grade; LDA: linear discriminant analysis; LG: low grade; n/a: not available; NIRS: near-infrared spectroscopy; OLS: ordinary least squares regression; PCA: principal component analysis; PDD: photodynamic diagnosis; PLS: partial least squares; SERS: surface-enhanced Raman spectroscopy; SVM: support vector machines; TURBT: transurethral resection of bladder tumour.

85% and specificity of 97% in distinguishing malignancy from cystitis and normal tissue[10].

Quality assessment and risk of bias

All articles were evaluated for risk of bias and concerns regarding applicability using the QUADAS-2 quality assessment tool (**Figure 2**). Up to 45% and 50% of the studies showed a high risk of bias regarding patient selection and index test, respectively. This was predominantly due to non-random patient selection and knowledge of reference standard results prior to interpreting the index test.

Discussion

Evolution of spectroscopy in clinical practice

Since the first ex vivo RS study by Crow et al. (2004) using frozen tissue (**Figure 3**), significant technological advancements in optoelectronics, computational capacity, and machine-learning data analysis techniques have facilitated rapid and real-time applications[24]. In the first in vivo study, Draga et al. (2010) introduced a fiber optic probe with a 2.1-mm external diameter via a cystoscope to acquire RS measurements immediately before TURBT[19]. Their algorithm achieved a sensitivity of 85% and a modest specificity of 79% in distinguishing BCa from normal tissue, thus highlighting the challenges in clinical translation for RS.

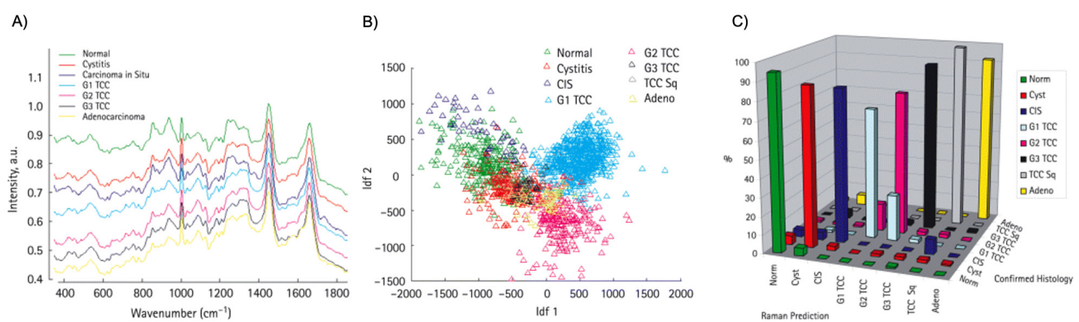
Subsequent RS studies have implemented hardware and software improvements to optimize diagnostic accuracy. Barman et al. (2012) introduced a confocal fiber optic probe that limited sampling to 300 µm. By suppressing spectral information from deeper tissue layers beyond the region of interest, diagnostic accuracy improved to 86% sensitivity and 100% specificity[25]. Following a similar principle of shallow tissue sampling, Stomp-Agenant et al. (2022) developed a superficial fiber optic probe with a measuring depth of 200 µm. This significantly reduced the signal-to-noise ratio compared to a regular probe, improving accuracy to 90% sensitivity and 87% specificity[18].

In addition to the hardware improvements described, advancements in computational capacity and machine-learning data analysis techniques continue to enhance the diagnostic accuracy of spectroscopy. Chen et al. (2018), analyzed 32 snap-frozen bladder specimens using a fiber optic probe, similar to previous studies, but combined PCA with artificial neural network (ANN) modelling to achieve a sensitivity of 98% and specificity of 96% in detecting high-grade BCa. ANN is a powerful, self-adaptive, and data-driven pattern recognition method capable of capturing non-linear characteristics of the data[26]. After comparing ANN with other popular classifications methods such as linear discriminant analysis (LDA) or support vector machines (SVM) in dozens of studies, the authors noted that ANN constantly outperformed other techniques.

While no single spectroscopy technique has proven to be perfect, a multimodal approach is likely to be required. RS has been combined with a concurrent diagnostic method in 3 studies, including photodynamic diagnosis[19], optical coherence tomography[27], and fluorescence and diffuse reflectance[28]. Although a multimodal approach improved accuracy up to 90%[28], the additional time and resource expenditure is significant, limiting this approach in a clinical setting.

FIGURE 3.

Figures from the first ex vivo Raman Spectroscopy study by Crow et al. (2004) using frozen tissue. **A)** The mean Raman spectra measured for each of the pathological groups. **B)** Scatter plots of the scores of linear discriminant function 1 vs. 2 showing clustering in the eight-group algorithm. **C)** The prediction power of the eight-group diagnostic algorithm demonstrating a sensitivity and specificity of 93% and 98%, respectively. (Reproduced with permission.[24])



Adeno: adenocarcinoma; a.u.: absorption units; CIS: carcinoma in situ; Cyst: cystitis; Idf; linear discriminant function; Sq: squamous; TCC: transitional cell carcinoma.

Comparison of VS techniques

In the context of BCa diagnosis, RS has received the most attention in recent years. A systematic review of 9 original studies conducted between 2004 and 2015 demonstrated an impressive pooled diagnostic sensitivity of 94% and specificity of 92%[29]. However, the studies included in this meta-analysis were highly heterogeneous in terms of sample type, instrument used, excitation wavelength, and algorithm for analysis. Some studies used snap-frozen or formalin-fixed paraffin-embedded (FFPE) tissue, while others used cell lines, peripheral blood, or urine.

There has been great interest in using RS to analyze urine samples for molecular signatures associated with BCa to develop a truly non-invasive screening test. Huttanus et al. (2020) developed an RS-based chemometric urinalysis (Rametrix) as a direct method for screening urine samples. Using a model built with 22 principal components, BCa was detected with 82.4% sensitivity and 79.5% specificity[30]. The reduced accuracy of this label-free method could be attributed to the diversity of urine composition, concentration, and pH[31]. With the surface enhancement provided by SERS, greater diagnostic accuracy could be achieved, as demonstrated a recent study by Hu et al. (2021) with 100% sensitivity and 98.85% specificity[32].

FT-IR analysis of bladder washings has also been proposed as a sensitive, rapid, non-destructive, and operator-independent analytical diagnostic method for BCa compared with traditional urine cytology. In a study by Gok et al. (2016), bladder washings were analyzed from 136 patients, demonstrating a sensitivity of 100% but modest specificity of 73.5%. Interestingly, traditional urine cytology had a sensitivity of only 45% on the same specimens in this study[33].

The combination of FT-IR with microscopy has also led to the development of IR imaging. Studies have demonstrated accuracies > 90% compared with immunohistochemical (IHC) diagnostics by pathologists[34]. However, clinical translation of this powerful integrated technique has been hindered by long measuring times and complex FT-IR setup requiring liquid nitrogen cooling. Quantum-cascade laser (QCL)-based microscopes have shown promise in overcoming these limitations, enabling IR imaging to be performed within minutes. Kuepper et al. (2018) demonstrated that QCL-based IR imaging could identify colorectal cancer in the same time frame as a frozen thin section diagnosis by pathologists, boasting a sensitivity of 96% and specificity of 100%[34].

Limitations of evidence and review process

Despite the high levels of diagnostic accuracy achieved with VS recently, this review has identified several limitations that indicate the need for further work before the clinical use of VS as a minimally invasive tool for cancer investigation. The inclusion criteria of this review did not impose limitations on sample size to provide a broader overview of all available literature on VS. As a result, one-third of studies included had fewer than 20 participants, offering a low level of evidence with significant heterogeneity in study design. Furthermore, variation in tissue sample preparation techniques, pathological grouping, and data analysis make direct comparison between studies difficult. This prevents any meaningful pooling of results through meta-analysis to obtain statistical estimates of overall diagnostic accuracies.

The reporting methods of the included studies were inconsistent and often incomplete. While many studies often reported sensitivity and specificity, they often omitted reporting accuracy and AUC, or vice versa. Only 1 study reported all 4 of measures of accuracy[25]. The use of the term "optimal" sensitivity in some studies raises concerns about reporting bias, as the authors may have been selecting the best results for reporting. Concerningly, none of the studies provided complete data for all key areas of diagnostic accuracy: true and false positivity and negativity, sensitivity, specificity, and positive and negative predictive values. It is paramount to publish larger studies that comprehensively report these values to further evaluate spectroscopy.

Implications on clinical practice and challenges for future research

While many of the studies included in this review analyzed ex vivo bladder specimens, the true potential of non-invasive VS lies in its application in a real-time in vivo setting. This will undoubtedly depend upon the development and optimization of fiber optic probes that can be introduced via the urologist's everyday cystoscope. This trend is already evident in the studies included in this review, with 4 of the 5 studies published in the past 5 years using a fiber optic probe[18,26,28,35]. Another major challenge is the presence of fluids such as urine or glycine, which can interfere with the spectroscopic signal and reduce specificity. More in vivo research is needed to evaluate the feasibility of VS in the operating theatre. The results of a phase 1 trial by Hermann et al. are awaited (ClinicalTrials.gov identifier: NCT05124106), as the study utilizes fiber optic probes to take RS measurements inside the bladder of 30 patients.

It is noteworthy that no studies using NIRS to analyze BCa were included in this review, despite the successful use of this technique in evaluating prostate cancer, breast cancer, and cardiac fibrosis specimens[36–38].

Recent improvements in machine-learning analytical techniques have led to substantial progress in research and industry[11,12]. NIRS has the potential to provide real-time molecular data, analogous to handheld ultrasound devices, with low computational requirements (6 Kb per spectrum), making it possible to be performed on mobile devices in line with the evolution toward ambulatory and personalized care[38]. Compared to the aforementioned techniques, NIRS spectra can be obtained from greater sample thickness, allowing for easier sample handling, and it is fast without the need for a laser, unlike RS. Additionally, near-infrared light penetrates deeper into human tissues, causing less photodamage and safer tissue probing[39]. Considering that NIRS and RS provide complementary information when analyzing the same sample, combining the 2 techniques in a multimodal approach could potentially further enhance diagnostic accuracy.

With ongoing advancements in spectroscopy technology, machine-learning analytical techniques, and multimodal approaches to improve accuracy, VS offers several potential advantages over standard histopathology: rapid, label-free, and operator independent. When used in conjunction with fiber optic probes in endoscopy, it may help reduce cases of incomplete tumour resection and lower the risk for recurrence. It can serve as a tool to aid clinical decision-making in real time, providing a quick and safe assessment of stage and grade, allowing urologists to reduce over- or under-treatment of BCa. In an increasingly frail population with rising anticoagulant use, these improvements could reduce adverse events related to surgery and expedite the staging and grading of urothelial cancer of the bladder.

Conclusions

Although VS is a mature technology in analytical chemistry, its use in medical diagnostics is still in its infancy. Recent advances in technology and computing power and reductions in equipment costs and size have facilitated a shift in focus from the laboratory to the bedside. As fiber optic probes for spectroscopy become commercially available, their use in combination with a conventional cystoscope opens up the exciting possibility for real-time diagnostic imaging of BCa. RS has demonstrated high levels of diagnostic accuracy, which continue to improve with advancements in SERS. However, studies are small and highly heterogeneous. Larger spectroscopy studies with robust reporting methods and a multimodal approach are needed to assess not only the overall diagnostic accuracies but also the optimal utilization of this emerging technology. Ongoing research into modalities such as SERS and NIRS holds great promise, making spectroscopy an exciting and dynamic field in urological diagnostics with the potential to enhance intraoperative decision-making.

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