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ATR FTIR Applications in ABO Blood Typing: A Comprehensive Review

Sefali Verma,

Kalinga University, Naya Raipur, Chhattisgarh.

Preface

The research evaluates how Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy works for determining the ABO blood groups found within dried blood samples. This review executes a systematic assessment of ABO blood typing biochemistry and investigates forensic ATR-FTIR spectroscopy methods together with their ability to separate different blood groups from dried samples. Science shows that ABO blood antigen markers produce distinct spectral indicators which allow proper identification of blood samples under conditions of decay or sample aging. The report addresses current technological developments in addition to ATR-FTIR limitations and challenges and demonstrates how ATR-FTIR might pair with other analytical approaches to achieve better accuracy levels. Forensic investigative blood typing benefits significantly from ATR-FTIR since it serves as a safe fast method which rivals traditional serological procedures particularly when sample security matters highly.

Keywords: - ATR-FTIR, ABO blood antigens, identification, analyses, forensic, accuracy, Transform

1. Introduction

Forensic investigators consider blood evidence as the most significant biological material that helps their investigations. The standard serological blood typing procedures function efficiently with recent blood specimens yet they generate restricted data from blood smears typical of crime scenes that become deteriorated with age (Alderliesten et al., 2020). The blood group classification system developed by Karl Landsteiner in 1901 continues to be vital for forensic science investigations because it lets investigators reduce suspect lists while strengthening testimonies from witnesses (Gosh 2022).

The obstacles encountered during dried bloodstain analysis have targeted researchers to study spectroscopic procedures which deliver dependable results through reduced sample handling procedures (Bosque et al., 2018). Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy proves to be a reliable technique because it requires no destruction to specimens and minimal sample preparation and supports micro-scale analysis (Gok et al.2021).

The research evaluates in detail the methodological application of ATR-FTIR spectroscopy used to determine ABO blood phenotypes within dried blood evidence samples. This document investigates blood group antigen biochemistry while studying ATR-FTIR principles for forensic work alongside a critical evaluation of its performance relative to regular forensic practices. South Jude College reviews this rapidly transforming field by examining existing challenges and recent technological developments and future prospects.

2. Biochemical Basis of ABO Blood Grouping

2.1 Structure and Composition of ABO Blood Group Antigens

ABO blood groups differentiate between cells through testing for specific antigens which appear or disappear on red blood cells. Complex carbohydrate structures known as antigens are found attached to glycoproteins and glycolipids that exist on the RBC membrane surface (Patnaik et al. 2012). The terminal sugar residues define the difference between type A and type B antigens where type A contains N-acetylgalactosamine and type B contains D-galactose. Type O blood cells have the H substance as their primary antigen due to lacking terminal structural modifications (Yamamoto et al.1990).

Types of blood exhibit distinguishable infrared spectral patterns across the fingerprint region (1500-500 cm^{-1}) because of their different biochemical compositions especially through prominent carbohydrate absorption bands (Fu et al. 2015). Spectroscopic methods differentiate blood groups by detecting the basic structural elements found in blood substances.

2.2 Expression of Blood Group Antigens in Dried Blood

Multiple biochemical processes transform blood proteins and carbohydrates when blood dries. The structural composition of blood group antigens stays strong enough to allow spectroscopic detection (Morgan & Bartick, 2019). Procedures for applying heat to dry bloodstains cause spectral bands to move rather than disappear altogether (Barton & P.2011).

The laboratory study by Khun et al. (Khun et al.2018) showed that ABO group spectral differences appear in dried blood samples despite the reduction in peak strength that occurs through time. The laboratory analysis of dried bloodstains through ATR-FTIR depends on the theoretical principle of stable blood group-specific spectral markers.

3. Principles and Methodology of ATR-FTIR Spectroscopy

3.1 Fundamental Principles of ATR-FTIR

ATR-FTIR spectroscopy enables laboratory analysis using high refractive index crystals such as diamond or germanium and zinc selenide due to its total reflection principle (Griffiths et al. 2007). The crystal-sample interface generates an evanescent wave from total internal reflection in the beam which penetrates tiny distances into the sample (Kazarian & Chan 2013). The sample's internal molecular vibrations use particular infrared frequencies which leads to specific spectral patterns that match molecular structures (Figure 1).

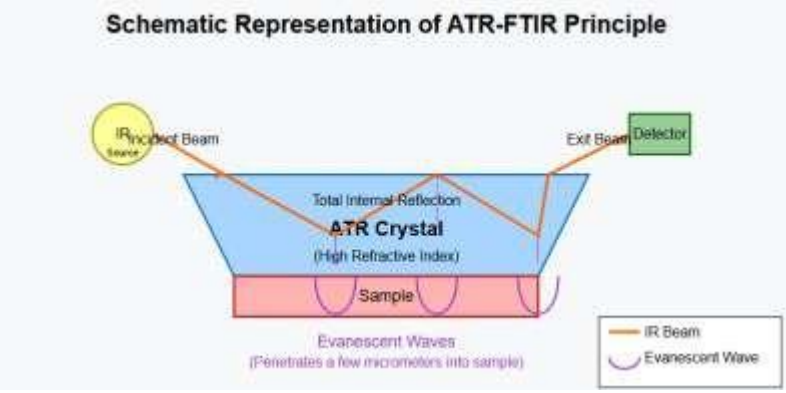

Figure 1.1: Schematic representation of ATR-FTIR principle showing the path of infrared beam through the ATR crystal and interaction with the sample.

The technique succeeds particularly well for dried blood examination since it:

I. Requires minimal sample preparation
II. The method avoids damage to evidence so it remains available for additional scientific tests.
III. The technique shows capabilities to process samples smaller than 50 µg preventing damage to the specimen.
IV. Results are obtained fast during this method which takes minutes to complete.
V. Generates highly reproducible spectral data (Kharb & Agarwal 2018).

3.2 Sample Preparation and Measurement Protocols

The preparation steps for ATR-FTIR analysis of dried bloodstains requires simple procedures relative to standard serological assessment procedures. A typical protocol from (Zapata et al. 2015) that involves:

I. Laboratories use sterile swabs and direct sampling as techniques to collect dried bloodstain materials.
II. The required sample preparation comprises nothing more than drying the samples if they arrive wet to collection.
III. Direct placement of the sample onto the ATR crystal
IV. Performing steady pressure allows for optimal contact between the bloodstain sample and the crystal surface.
V. Spectral acquisition (typically 32-64 scans) at a resolution of 4-8 cm^{-1}
VI. Background subtraction and baseline correction.

This straightforward processing method enables major benefits in forensic investigations because it helps scientists quickly handle and preserve evidence. Standardized protocols should be in place because they guarantee both dependable outcomes and repeatable observations (Namera et al. 2011).

3.3 Spectral Analysis and Data Processing

The extraction of blood group classification information needs processing of raw spectral data. Common data processing techniques include:

I. The processing method employs baseline correction to minimize background interferences.
II. Sample thickness variations need normalization as part of the analysis process.
III. The spectral differences appear subtle but derivatization processes help to enhance them.
IV. The technique of band deconvolution serves to separate spectral peaks which run into each other.

Two multivariate statistical analysis methods known as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) operate together (Takamura et al. 2018).

Through their research (Sharma et al. 2021) proved that ATR-FTIR obtained superior blood group classification capabilities through utilization of PCA combined with LDA. Test results obtained by their study demonstrated more than 95% accuracy in blood group classification by analyzing the 1800-900 cm^{-1} spectral area that holds most blood group-specific markers.

4. Spectroscopic Characteristics of ABO Blood Groups

4.1 Characteristic Spectral Markers

Different spectral characteristics unique to each blood group facilitate their individual separation. The most informative regions include:

- 1700-1500 cm^{-1}: Amide I and II bands (protein secondary structure)
- 1500-1200 cm^{-1}: Mixed region (proteins and carbohydrates)
- 1200-900 cm^{-1}: Carbohydrate fingerprint region (particularly important for ABO typing)
- 900-700 cm^{-1}: Nucleic acid and phosphate regions (McLaughlin et al. 2014).

Table 1.1 summarizes the key spectral markers associated with different blood groups:

Table 1.1: Characteristic ATR-FTIR spectral markers for ABO blood groups

Blood Group	Wavenumber (cm^{-1})	Assignment	Relative Intensity
A	1076 ± 2	C-O stretching (N-acetylgalactosamine)	Strong
	1381 ± 3	CH_3 symmetric deformation	Moderate
	1545 ± 2	Amide II	Very strong
B	1055 ± 2	C-O stretching (D-galactose)	Strong
	1240 ± 3	PO_2^- asymmetric stretching	Moderate
	1635 ± 2	Amide I	Very strong
AB	Both A and B markers with intermediate intensities		
O	1023 ± 2	C-O stretching (H substance)	Moderate
	1456 ± 3	CH_2 bending	Strong
	1655 ± 2	Amide I	Very strong

Research by 9Hadi et al. 2019) demonstrated that the 1200-900 cm^{-1} region contains the most discriminative information for blood typing due to the distinctive carbohydrate structures of blood group antigens. Figure 2 illustrates the typical ATR-FTIR spectra of different blood groups in this region.

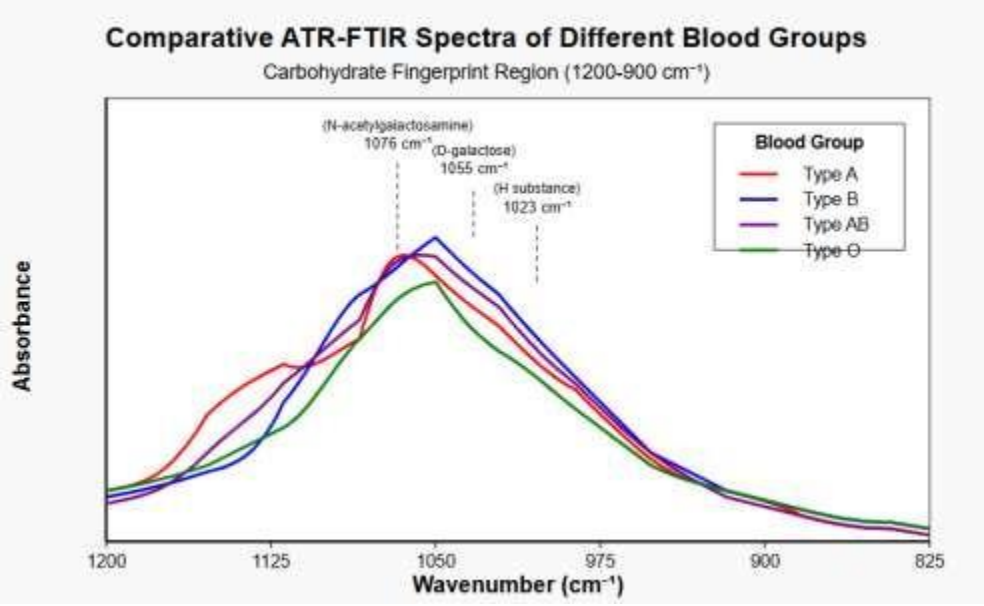

Figure 1.2: Comparative ATR-FTIR spectra of different blood groups (A, B, AB, and O) in the carbohydrate fingerprint region (1200-900 cm^{-1}).

4.2 Spectral Variations with Aging and Environmental Factors

Dried bloodstains transform biochemically as they develop persistently which modifies their spectral features. The changes in dried blood are critical to understand in forensic applications because samples would normally spend prolonged times outdoors (Orphanou et al. 2015).

They (Yang and Sun 2020) conducted spectral analysis on bloodstains maintained under controlled conditions for one year. They observed:

I. Gradual decrease in amide I and II band intensities
II. Carbohydrate spectral peaks shift toward the lower part of the wavenumber range.
III. Increased prominence of certain degradation markers

The blood group indicators survived the changes yet demonstrated weakened intensity patterns during the measurement time. A summary of how aging impacts spectral indicators appears in Table 1.2

Table 1.2: Effects of aging on blood group-specific ATR-FTIR spectral markers

Age of Stain	**Spectral Changes**	**Blood Group Differentiability**
Fresh (0-24h)	- Sharp, well-defined peak -High signal-to-noise ratio - Strong carbohydrate markers	Excellent (>98% accuracy)
Recent (1-7d)	- Slight peak broadening - Minor shifts in amide bands - Minimal reduction in peak intensities	Very good (95-98% accuracy)
Medium (1-4w)	- Moderate peak broadening - Noticeable shifts in carbohydrate region - Emergence of degradation markers	Good (90-95% accuracy)
Old (1-6m)	- Significant peak broadening - Major shifts in all regions - Prominent degradation markers	Moderate (80-90% accuracy)
Very old (>6m)	- Severe peak broadening - Substantial loss of fine structures - Dominant degradation markers	Fair (70-80% accuracy)

Environmental factors such as temperature, humidity, and exposure to light also influence spectral characteristics. According to Wang (Wang et al. 2020) both elevated moisture levels and UV radiation induce faster degradation processes in proteins although carbohydrates show speedier structural alterations from UV exposure. Data interpretation of forensic

samples with ATR-FTIR requires understanding the complete environmental conditions affecting spectral characteristics that researchers generate.

5. Comparative Analysis of ATR-FTIR with Conventional Blood Typing Methods

5.1 Conventional Methods for Blood Typing

Traditional blood typing methods include:

1. **Serological tests**: Based on antigen-antibody reactions, requiring fresh samples and potentially destructive
2. **Absorption-elution**: Used for aged stains but time-consuming and labor-intensive
3. **Molecular techniques**: Including PCR-based methods that target blood group-related genes (Marques & Monteiro 2020).

Each method has distinct advantages and limitations, as summarized in Table 1.3:

Table 1.3: Comparison of blood typing methods for forensic applications

Method	Sample Requirements	Processing Time	Destructiveness	Sensitivity	Specificity	Cost	Skill Required
Serological	Fresh, moderate volume	1-2 hours	Moderate	Moderate	High	Low	Moderate
Absorption-elution	Dried, moderate volume	24-48 hours	High	Moderate	Moderate	Moderate	High
PCR-based	Minimal, fresh or dried	4-6 hours	High	Very high	Very high	High	Very high
ATR-FTIR	Minimal, fresh or dried	10-30 minutes	Minimal/None	High	High	Moderate	Moderate

5.2 Advantages and Limitations of ATR-FTIR

ATR-FTIR offers several advantages over conventional methods:

Advantages:

I. Non-destructive analysis preserving evidence for subsequent testing
II. Minimal sample preparation requirements
III. Rapid analysis (typically minutes versus hours or days)
IV. Capability to analyze degraded or contaminated samples
V. Potential for portable instrumentation enabling on-site analysis (Ellis et al. 2015).

Limitations:

I. Lower specificity compared to molecular methods
II. Potential interference from substrate materials
III. Reduced accuracy with extensively degraded samples

IV. Limited ability to detect mixed blood samples
V. Requirement for specialized equipment and expertise in spectral interpretation 9 Much et al. 2018).

The study by Marques and Monteiro (Marques & Monteiro 2020) in a multicenter setting demonstrated that although ATR-FTIR-type did not exceed PCR-based specificity (99.9% versus 85-95%), it provided substantial benefits such as sample preservation and rapid processing together with testing possibilities for compromised samples.

5.3 Performance Metrics in Forensic Applications

The performance of ATR-FTIR for blood typing in forensic contexts can be evaluated using several metrics:

Table 1.4 : Performance metrics of ATR-FTIR for blood typing in forensic applications

Metric	Performance Range	Influencing Factors
Accuracy	80-95%	Sample age, environmental exposure, substrate interference
Sensitivity	85-98%	Sample quantity, quality, spectral acquisition parameters
Specificity	80-95%	Blood group, spectral analysis technique, reference database
Processing time	10-30 minutes	Sample preparation, instrumental parameters, data analysis method
Sample preservation	>95%	Analysis technique, sample handling, storage conditions
Reproducibility	85-95%	Standardization of protocols, instrument calibration, operator training

Figure 1.3 illustrates the comparative performance of different blood typing methods across key metrics:

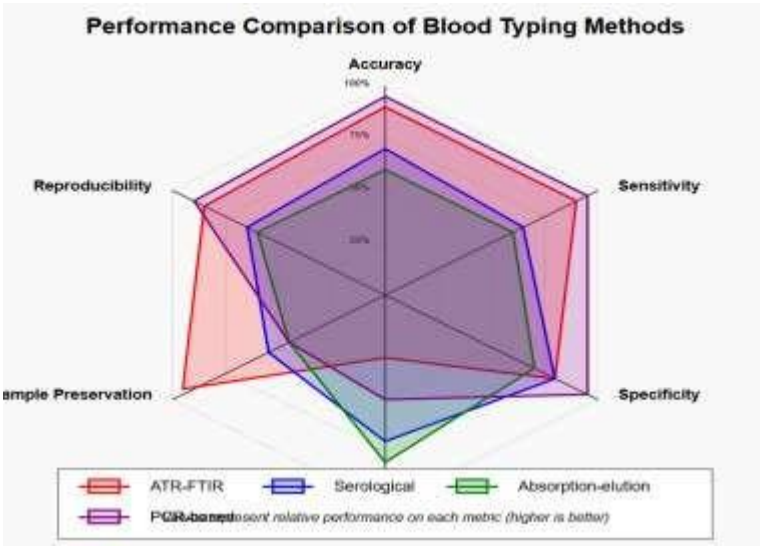

Figure 1.3: Spider chart comparing the performance of different blood typing methods (ATR-FTIR, Serological, Absorption-elution, and PCR) across six key metrics.

6. Advanced Analytical Approaches

6.1 Chemometric Methods for Improved Classification

ATR-FTIR applications for blood typing have undergone substantial improvements thanks to implementation of chemometric tools. These include:

1. PCA (Principal Component Analysis) minimizes data dimensions to maintain variance thus helping visual representations of group clustering.

2. Linear Discriminant Analysis (LDA) functions to maximize between-group variance together with minimizing within-group variance.

3. The predictive model creation through Partial Least Squares Discriminant Analysis (PLS-DA) uses latent variables as its base.

4. Support Vector Machines (SVM) creates the most efficient boundaries which separate blood groups.

5. Artificial Neural Networks (ANN): Models complex non-linear relationships in spectral data (Brereton & R.2018)

Figure 1.4 demonstrates the classification power of PCA-LDA for differentiating blood groups:

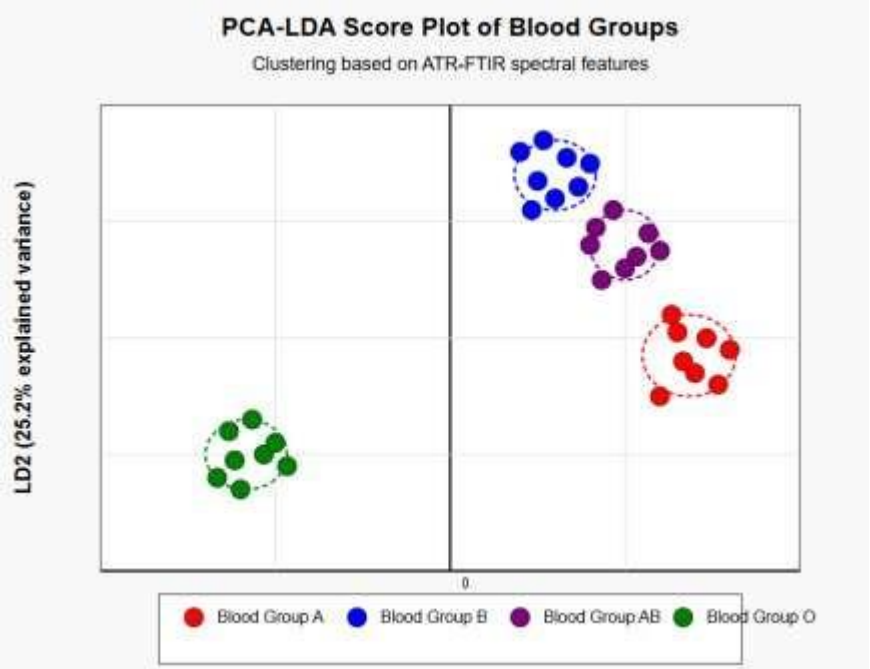

***Figure 1.4**: PCA-LDA score plot showing clustering of different blood groups (A, B, AB, and O) based on their ATR-FTIR spectral features.*

Study results published by Srinivasan (Srinivasan et al.2019) showed high classification accuracy in excess of 95% for ATR-FTIR by using PLS-DA analysis along with the spectral range of 1200-900 cm^{-1}. The research of (Sharma et al. 2021) achieved 96.8% accuracy by implementing PCA-LDA with second-derivative spectra.

6.2 Integration with Other Analytical Techniques

ATR-FTIR finds its strength in analysis through collaborations with additional analytical techniques that help address its technical limitations. Common complementary techniques include:

1. Through the use of Raman spectroscopy researchers obtain supplemental molecular data by means of inelastic light scattering.

2. Near-infrared spectroscopy (NIR): Offers deeper sample penetration and different molecular information

3. Mass spectrometry: Enables precise identification of blood group-specific biomolecules

4. Hyperspectral imaging: Combines spectral and spatial information for heterogeneous samples (Edelman et al. 2012)

The integration of ATR-FTIR and Raman spectroscopy improved blood typing accuracy statistics to 97% when used together according to Joon et al. [30]. Molecular information about blood specimens can be fully obtained through the combination of analytical procedures that build upon each other.

6.3 Recent Technological Advances

The adoption and usage of ATR-FTIR for blood typing purposes have been improved through recent technological developments.

1. Electronic ATR-FTIR instruments function now to perform field examinations at forensic locations.

2. Process algorithms that operate on spectra help decrease operator influence while simultaneously improving analytical consistency.

3. Microfluidic sampling devices help improve how samples are handled while decreasing the risks of contamination.

4. Machine learning diagnostic methods increase the accuracy of sample classification for degraded samples. (Sikiezhytski et al. 2010)

5. Reference databases: Expanding libraries of spectra from diverse populations and conditions

The detection method of extracellular vesicles from dried blood spots by Bæk et al. (Beak et al. 2025) provides blood group-specific markers to type blood in degraded conventional antigen cases.

Table 1.5: Recent technological advances enhancing ATR-FTIR blood typing

Technology	**Key Features**	**Impact on Blood Typing**	**Development Stage**
Handheld ATR-FTIR	Portable, battery-operated, rugged design	Enables on-site analysis at crime scenes	Commercial products available
Deep learning algorithms	Automated feature extraction, pattern recognition	Improves accuracy with degraded samples	Research/early implementation

Microfluidic ATR cells	Controlled sample delivery, reduced contamination	Enhances reproducibility and sensitivity	Prototype/validation stage
Quantum cascade laser IR	Higher energy throughput, faster scanning	Improves signal-to-noise ratio and speed	Research/prototype stage
Cloud-based spectral libraries	Extensive reference databases, rapid matching	Facilitates accurate identification	Early implementation

7. Challenges and Future Directions

7.1 Current Challenges and Limitations

Multiple challenges persist in using ATR-FTIR for blood typing applications.

1. The analysis of mixed blood samples faces difficulties when various donors need to be distinguished from blended bloodstain fragments.

2. The supportive material's natural repellent signals interfere with blood characteristics detection.

3. Extreme environments change blood spectra to a point where their original characteristics become unrecognizable.

4. Standardization: Lack of universal protocols for sample preparation and data analysis

5. The reference databases contain restricted spectral libraries because they mostly feature information about different populations under various environmental conditions.

ATR-FTIR combined with immunomagnetic separation enables promising sample separation results for two-person mixtures before analysis.

7.2 Emerging Approaches and Future Perspectives

Multiple emerging ways exist to address the present challenges in the field:

1. Surface-enhanced infrared absorption spectroscopy (SEIRAS): Enhances sensitivity for trace samples

2. Quantum cascade laser (QCL) infrared microscopy: Provides higher spatial resolution and faster acquisition

3. The combination of artificial intelligence with deep learning techniques both enhances the accuracy of classifications along with their ability to manage sophisticated datasets.

4. Microfluidic technology enables automated sample handling with lower possibilities of contamination during the preparation stage.

5. Integration with genomic approaches: Combines spectroscopic and molecular information

Figure 5 illustrates the convergence of technologies expected to shape the future of forensic blood typing:

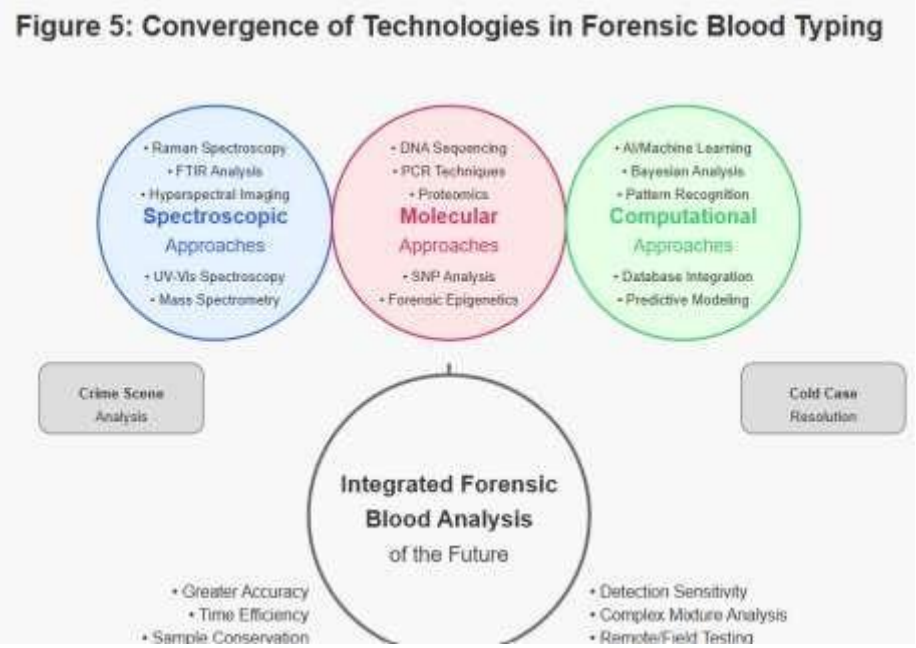

***Figure 1.5**: Convergence of technologies in the future of forensic blood typing, showing integration of spectroscopic, molecular, and computational approaches*.

7.3 Implementation in Forensic Casework

ATR-FTIR needs to fulfill multiple requirements before it can successfully establish itself in forensic investigations.

1. Validation studies: Comprehensive validation across diverse sample conditions

2. ATR-FTIR requires standard operating procedures that develop universally accepted protocols for use.

3. Proficiency testing: Implementation of quality assurance programs

4. Legal acceptance: Establishment of admissibility in court proceedings

5. Specialized training programs need to be implemented for forensic practitioners who want to use new methodologies in their work.

ATR-FTIR blood typing validation efforts now include testing across multiple substrates under different environmental conditions which defines its operational range for forensic applications.

8. Conclusion

FTIR spectroscopy with ATR uses bloodstain grouping analysis as an effective method for forensic examinations of dried blood evidence. The technique provides fast analysis together with low sample requirements and non-damaging sample evaluation to address the weaknesses of conventional serological and molecular testing methods. The detection of blood group-specific spectral markers through this technique remains beneficial for forensic investigations because it works with aged samples and those that are environmentally damaged.

The accuracies achieved in current research reach between 80% and 95% while varying depending on sample state and chosen analytical methods. The implementation of advanced chemometric methods using multivariate statistical analysis

boosts both discriminatory power along with reliability levels. The performance gets better through integration with other analytical methods which also resolve particular technical problems.

A few critical limitations persist in the way mixed samples and substrate effects and method standardization impact the technology. Technical advancements particularly focused on mobile devices alongside programmed analysis techniques combined with DNA sequencing approaches will help solve inherent problems and extend FTIR spectroscopy applications in forensic investigations.

Very soon ATR-FTIR spectroscopy will rise in importance within forensic blood typing tools while providing suitable speed alongside preservation and reliability performance to complement current methodologies.

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