

**DIFFERENTIATION OF HUMAN BLOOD FROM ANIMAL BLOOD -
A FORENSIC STUDY**

RUNNING TITLE : Analysing the differences in human and animal blood

Sushmitha V

Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
E-mail: 152001084.sdc@saveetha.com

V. Vishnu Priya

Professor
Department of Biochemistry, Saveetha Dental college and Hospitals
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
E-mail: vishnupriya@saveetha.com

Abirami Arthanari

Lecturer
Department of Forensic Odontology, Saveetha Dental college and Hospitals
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
E-mail: abiramia.sdc@saveetha.com

Gayathri.R

Associate Professor
Department of Biochemistry, Saveetha Dental college and Hospitals
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
Email: gayathri.sdc@saveetha.com

Kavitha.S

Lecturer
Department of Biochemistry, Saveetha Dental college and Hospitals
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
E-mail: kavithas.sdc@saveetha.com

Reshma PK

Lecturer
Department of Oral Pathology, Saveetha Dental college and Hospitals
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
Email: reshmapk.sdc@saveetha.com

Corresponding author

V. Vishnu Priya

Professor
Department of Biochemistry, Saveetha Dental college and Hospitals
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077
Tamilnadu, India
Email: vishnupriya@saveetha.com

ABSTRACT:

Introduction

Identifying blood samples is a crucial step of a forensic investigation, especially of major and violent crimes. Animal and human blood look alike to a naked eye but its biochemical aspects are different. It is extremely important to analyse whether evidentiary blood stains are of human origin or not in a forensic investigation. Thus differentiating blood samples is important in a forensic investigation.

Aim

To differentiate human blood from animal blood in the given blood samples for forensic purposes.

Materials and methods

Blood samples of each human and animal (Wistar albino rat) was collected from clinical lab and animal house respectively. The difference in the constituents of blood and various cell counts was analysed with the help of a simple microscope and CBC (complete cell count) machine. Statistical analysis was done using SPSS software version 23.0 and corresponding graphs were plotted.

Results

A significant difference was observed in the cell counts and blood group type. The platelet and RBC count of that of wistar albino rats was higher than humans. There were similar haemoglobin levels. The animal blood sample taken did not fall under the ABO blood grouping unlike that of the humans.

Conclusion

These differences observed are helpful in the differentiation of human and animal blood for forensic purposes. Usage of advanced technologies will make this differentiation even more easier.

Keywords

Innovative technique, Blood, Electron microscope, Forensics, Haemoglobin, Hemocytometer, Wistar albino rat, novel method.

INTRODUCTION:

A quicker and accurate way to differentiate human blood from animal blood can prove key in crash investigations. Techniques which can rapidly tell the difference between human blood and that of nearly a dozen animal species without destroying the sample is of great importance in forensics. (1)

Under favourable conditions when the stains are not too old, it's easy to determine if its blood or something else by spectroscopic examination and by developing hematin crystals. (2) An example protocol for the examination of blood involves physical examination, preliminary, confirmatory tests which are specific to blood types are important. Discrimination between animal and human blood is very important in a hit and run case where the suspect may lie, where blood stains from the vehicle can be tested to determine the origin. (3) Was the first to report the use of vibrational spectroscopy for discrimination between human and animal blood sample.

The normal ranges of various cell counts for humans is, RBC- 4.2 to 5.9 million cells/cmm, WBC- 3.4 to 9.6 billion cells/L and platelet count should be 1,50,000 to 4,00,000 cells/cmm and haemoglobin level is 12 to 17g/dl. The normal range for animals is, RBC-8.5 to 10.5 million/cmm, Platelets count- 6,73,000 to 15,00,000 and haemoglobin level should be 11 to 15g/dl. The MCV (Mean corpuscular volume) for animal sample is around 60 to 70 fl and that of human sample is 80 to 95 fl.

Current methods to differentiate blood types include, extracting either a nuclear DNA or mitochondrial DNA profile from a blood sample which gives accurate results. (4) It is practically impossible to obtain and analyse blood samples from every known species, but if enough donors are available a universal statistical model for differentiating human and animal blood can be done.

Various research groups have demonstrated various methods, out of which Raman spectroscopy and Principal component analysis (PCA) were used with a confidence level of 99%. (5) Other groups have also used Partial least squares discriminant analysis (PLSDA), diffuse reflectance spectroscopy. (6,7). Our team has extensive knowledge and research experience that has translate into high quality publications (8),(9),(10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27)

. The main aim of this study is to show the basic differences between human and animal blood samples for forensic purposes.

MATERIALS AND METHODS:

Blood sample

Blood samples (n=6) of humans and animals (wistar albino rat) each used to study the differences in blood types. The human blood samples were collected irrespective of age, gender. All samples were refrigerated until used for analysis. EDTA was added to prevent the blood from coagulation.

Instruments and settings

The blood samples were collected from the clinical lab and BRULAC of Saveetha Dental College, Chennai. All cell counts for human blood samples were calculated using a CBC (complete blood count) machine. While that of animals were done manually, RBC counts were calculated using a hemocytometer, WBC using a counting chamber (a simple microscope was used) and haemoglobin count using a comparator box. Blood group identification was done by checking the agglutination of a sample with the corresponding antibody. (Antibody-antigen reaction-principle behind the test.)

Statistical analysis

The data obtained was analysed using SPSS software version 23.0. Cross tabs were made and the data was represented in the form of bar graphs.

RESULTS;

From the above data analysed we can see that, Figure 1 represent the platelet count for human and animal blood, the animal blood had a count of 1480×10^3 /microlitre and that of human was 216×10^3 /microlitre.

From Figure 2 we can see that the RBC count of human blood sample is 4.86×10^6 /microlitre and that of the animal is 10.2×10^6 /microlitre. From Figure 3 the Hb content of the human blood sample is 15.2g/dl and that of the animal blood sample is 12.2g/dl.

Figure 4 represents the blood group type of humans (ABO Type) and that the animal blood sample does not come under the ABO grouping. These are the inferences which show that there are significant differences between human and animal blood.

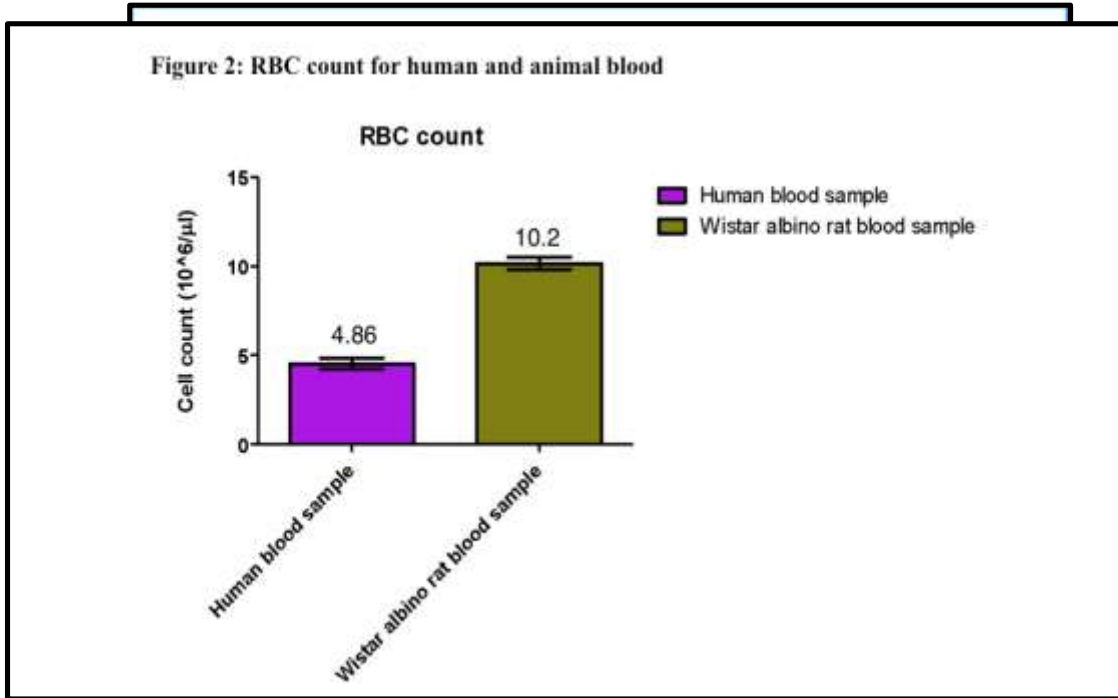


Figure 1: This bar graph represents the platelet count of the human and animal (wistar albino rat) blood sample. X axis represents the blood sample type and Y axis represents the cell count. In this graph orange colour represents the human blood sample type and green colour denotes the blood sample of wistar albino rat. The platelet count of the human and animal blood sample is 216×10^3 /microliter and 1480×10^3 / microliter respectively.

Figure 2: This bar graph represents the RBC count of the human and animal (wistar albino rat) blood sample. X axis represents the blood sample type and Y axis represents the cell count. In this graph purple colour represents the human blood sample type and green colour denotes the blood sample of wistar albino rat. The RBC count of the human and animal blood sample is 4.86×10^6 /microlitre and 10.2×10^6 /microlitre respectively.

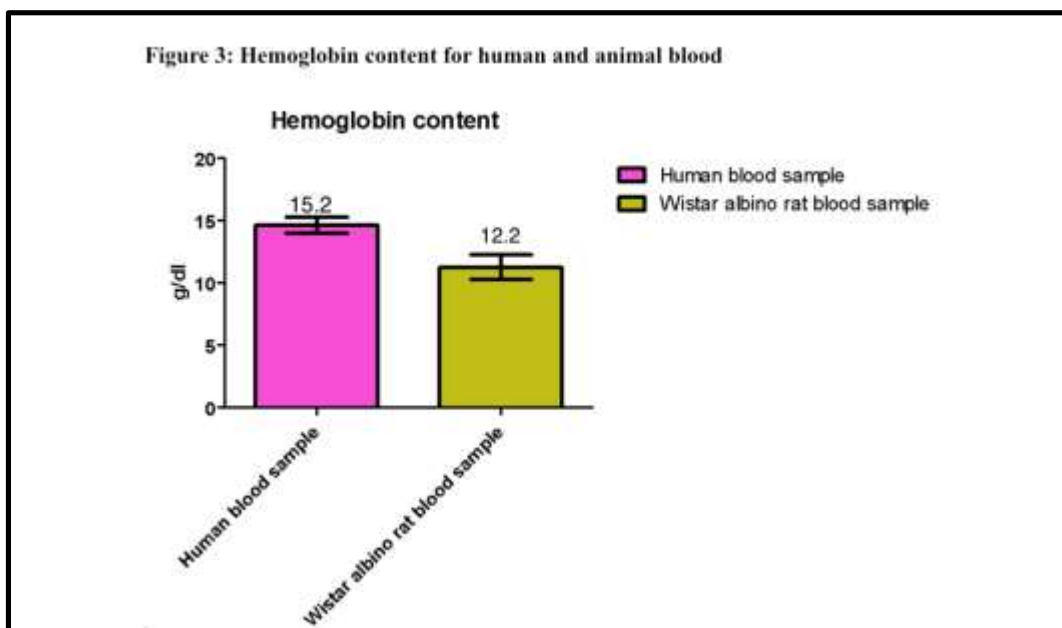


Figure 3: This bar graph represents the haemoglobin content of the human and animal (wistar albino rat) blood sample. X axis represents the blood sample type and Y axis represents the haemoglobin level. In this graph pink colour represents the human blood sample type and green colour denotes the blood sample of wistar albino rat. The haemoglobin level of the human and animal blood sample is 15.2 g/dl and 12.2 g/dl respectively.

| BLOOD SAMPLE | TYPE |
|---------------------|--|
| Human blood sample | B+ve (belongs to ABO blood grouping) |
| Animal blood sample | Does not come under ABO blood grouping |

Figure 4: This table represents the type of blood present in humans and animals. The human blood sample taken here is B+ve which belongs to the ABO blood grouping while that of the animal sample does not come under the ABO blood grouping.

DISCUSSION

From the present study, we can conclude that the animal blood samples (Wistar albino rat) have more RBC and platelet count than human blood samples. But the Hb level is quite less or almost similar which was the same according to the findings. (28) (29).

There is significant difference in the RBC counts of humans and wistar albino rat (higher than humans) similar to the previous studies (29,30) where there was a significant difference in RBC count-membrane related metabolic difference and concluded that human RBC banking cannot be done with that of wistar albino's, if used will lead to high haemolysis due to non compatibility.(31)

Even though both the blood samples look the same there was a biochemical difference in the count, types etc similar to the findings of (32). Where the differences in the biochemical aspects were considered to be chemical fingerprints which makes them unique. Animal blood did not come under the ABO, Rh blood grouping of humans similar to the findings of (33).

Even though there are differences, the basic constituents of blood remain the same, every blood has RBCs, WBCs, platelets, neutrophils, basophils etc which are similar to the previous literature. (34) There was a significant difference in the average diameter, volume, ratio of RBC volume:diameter in different animals which differentiated particle adhesion, laminar flow rate etc.

Few of the limitations of the study include not involving many types of animals, many blood samples of humans of different ethnic groups, and correlations of blood types. This study in the future when expanded to include

different types of animals, people from different ethnic groups, creating new correlations will have more accuracy and precision.

CONCLUSION;

There are both similarities and differences in the blood of humans and animals. These differences in the blood are very important in a forensic investigation. This study helped in analysing the basic differences in the blood types, which will be of great significance in the future of forensics. In depth study of the ultrastructure of cells and its components in future will help us to differentiate blood types more precisely which may help in depth forensic investigation.

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CONFLICT OF INTEREST:

The authors hereby declare that there is no conflict of interest in this study.

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AUTHORS CONTRIBUTION:

Sushmitha V : Contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

Dr. Vishnu Priya: Contributed in study design, guiding the research work, manuscript correction.

Dr. Abirami Arthanari, Gayathri.R, Kavitha.S, Reshma PK: Study design, statistical analysis, manuscript proofreading and correction.

REFERENCES;

1. Mistek-Morabito E, Lednev IK. Discrimination between human and animal blood by attenuated total reflection Fourier transform-infrared spectroscopy [Internet]. Vol. 3, Communications Chemistry. 2020. Available from: <http://dx.doi.org/10.1038/s42004-020-00424-8>
2. Khandasamy SR, Fikiet MA, Mistek E, Ahmed Y, Halámková L, Bueno J, et al. Bloodstains, paintings, and drugs: Raman spectroscopy applications in forensic science [Internet]. Vol. 8, Forensic Chemistry. 2018. p. 111–33. Available from: <http://dx.doi.org/10.1016/j.forc.2018.02.002>
3. Doty KC, Lednev IK. Differentiation of human blood from animal blood using Raman spectroscopy: A survey of forensically relevant species. *Forensic Sci Int*. 2018 Jan;282:204–10.
4. Kamanna S, Henry J, Voelcker N, Linares A, Kirkbride KP. “Bottom-up” in situ proteomic differentiation of human and non-human haemoglobins for forensic purposes by matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2017 Nov 30;31(22):1927–37.
5. Mistek E, Lednev IK. Identification of species’ blood by attenuated total reflection (ATR) Fourier transform infrared (FT-IR) spectroscopy. *Anal Bioanal Chem*. 2015 Sep;407(24):7435–42.
6. Zhang L, Zhou M, Li X-X, Li G, Lin L. Discrimination of human and nonhuman blood using visible diffuse reflectance spectroscopy [Internet]. Vol. 6, Anal. Methods. 2014. p. 9419–23. Available from: <http://dx.doi.org/10.1039/c4ay01673d>
7. Zhang L, Zhang S, Sun M, Wang Z, Li H, Li Y, et al. Blood species identification using Near-Infrared diffuse transmitted spectra and PLS-DA method [Internet]. Vol. 76, Infrared Physics & Technology. 2016. p. 587–91. Available from: <http://dx.doi.org/10.1016/j.infrared.2016.04.025>
8. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). *Artif Cells Nanomed Biotechnol*. 2019 Dec;47(1):3297–305.
9. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constituents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating Nrf2 signaling in human epidermal keratinocytes (HaCaT cells). *J Photochem Photobiol B*. 2019 Aug;197:111518.
10. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating

- the MMP signaling pathway [Internet]. Vol. 203, Journal of Photochemistry and Photobiology B: Biology. 2020. p. 111773. Available from: <http://dx.doi.org/10.1016/j.jphotobiol.2019.111773>
11. Babu S, Jayaraman S. An update on β -sitosterol: A potential herbal nutraceutical for diabetic management. *Biomed Pharmacother*. 2020 Nov;131:110702.
 12. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from *Albizia lebbek* on HCT-116 colon cancer cell lines. *Artif Cells Nanomed Biotechnol*. 2020 Dec;48(1):1206–13.
 13. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from *Trichosanthes kirilowii* in colon cancer cells through the induction of apoptotic pathway. *Artif Cells Nanomed Biotechnol*. 2019 Dec;47(1):3577–84.
 14. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. *Biomed Pharmacother*. 2018 Nov;107:1514–22.
 15. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: *Terfezia clavaryi*, *Terfezia boudieri*, and *Tirmania nivea* [Internet]. *Food Reviews International*. 2021. p. 1–20. Available from: <http://dx.doi.org/10.1080/87559129.2021.1889581>
 16. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. *Environmental Nanotechnology, Monitoring & Management*. 2020 Dec 1;14:100333.
 17. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitro and in vivo studies. *Arabian Journal of Chemistry*. 2020 May 1;13(5):5501–16.
 18. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. *Crit Rev Food Sci Nutr*. 2020 Oct 27;1–8.
 19. Barma MD, Muthupandiyani I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol*. 2021 Jun;126:105132.
 20. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent*. 2021 Mar;31(2):285–6.
 21. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent*. 2021 May;31(3):436–41.
 22. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from *Scutellaria baicalensis* on human osteoblast-like MG-63 cells [Internet]. Vol. 119, *Materials Science and Engineering: C*. 2021. p. 111656. Available from: <http://dx.doi.org/10.1016/j.msec.2020.111656>
 23. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. *Environ Toxicol*. 2021 Jun;36(6):1011–20.
 24. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of *Scutellaria barbata* and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). *Saudi J Biol Sci*. 2021 Jul;28(7):3633–40.
 25. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. *Saudi J Biol Sci*. 2021 Jul;28(7):3641–9.
 26. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Vol. 15, *Journal of Dental Sciences*. 2020. p. 562–3. Available from: <http://dx.doi.org/10.1016/j.jds.2020.02.009>
 27. Theertha M, Sanju S, Priya VV, Jain P, Varma PK, Mony U. Innate lymphoid cells: Potent early mediators of the host immune response during sepsis. *Cell Mol Immunol*. 2020 Oct;17(10):1114–6.
 28. McLaughlin G, Doty KC, Lednev IK. Raman spectroscopy of blood for species identification. *Anal Chem*. 2014 Dec 2;86(23):11628–33.
 29. Bian H, Wang P, Wang J, Yin H, Tian Y, Bai P, et al. Discrimination of human and nonhuman blood using Raman spectroscopy with self-reference algorithm. *J Biomed Opt*. 2017 Sep;22(9):1–7.
 30. Sajid J, Elhaddaoui A, Turrell S. Comparison of Various Vibrational Spectroscopic Techniques for the Study

- of Human Tissues [Internet]. *Spectroscopy of Biological Molecules: Modern Trends*. 1997. p. 463–4. Available from: http://dx.doi.org/10.1007/978-94-011-5622-6_208
31. Takamura A, Watanabe K, Akutsu T, Ikegaya H, Ozawa T. Spectral Mining for Discriminating Blood Origins in the Presence of Substrate Interference via Attenuated Total Reflection Fourier Transform Infrared Spectroscopy: Postmortem or Antemortem Blood? *Anal Chem*. 2017 Sep 19;89(18):9797–804.
 32. Zapata F, de la Ossa MÁF, García-Ruiz C. Emerging spectrometric techniques for the forensic analysis of body fluids [Internet]. Vol. 64, *TrAC Trends in Analytical Chemistry*. 2015. p. 53–63. Available from: <http://dx.doi.org/10.1016/j.trac.2014.08.011>
 33. Adili N, Melizi M, Belabbas H. Species determination using the red blood cells morphometry in domestic animals. *Vet World*. 2016 Sep;9(9):960–3.
 34. Zapata F, de la Ossa MÁF, García-Ruiz C. Differentiation of Body Fluid Stains on Fabrics Using External Reflection Fourier Transform Infrared Spectroscopy (FT-IR) and Chemometrics [Internet]. Vol. 70, *Applied Spectroscopy*. 2016. p. 654–65. Available from: <http://dx.doi.org/10.1177/0003702816631303>