Biomarker

In biomedical contexts, a **biomarker**, or **biological marker** is a measurable indicator of some biological state or condition. Biomarkers are often measured and evaluated using blood, urine, or soft tissues [1] to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[2] Biomarkers are used in many scientific fields.

Digital biomarkers are a novel emerging field of biomarkers, mostly collected by smart biosensors.[3] So far, digital biomarkers have been focusing on monitoring vital parameters such as accelerometer data and heartrate[4][5] but also speech.[6] Novel non-invasive, molecular digital biomarkers are increasingly available recorded by e.g. on-skin sweat analysis (*internet-enabled Sudorology*), which can be seen as **next-generation digital biomarkers**.[7] Digital biomarkers can be easily shared with the responsible physician, and novel diagnostics approaches can be developed using artificial intelligence.

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Medicine

Biomarkers used for medicine, or precision medicine, are a part of a relatively new clinical toolset. They are categorized in 3 primary ways according to their clinical applications. They are classified as molecular biomarkers, cellular biomarkers or imaging biomarkers. All 3 types of biomarkers have a clinical role in narrowing or guiding treatment decisions and follow a sub-categorization of being either predictive, prognostic, or diagnostic.

Predictive

Predictive molecular, cellular, or imaging biomarkers that pass validation can serve as a method of predicting clinical outcomes. Predictive biomarkers are used to help optimize ideal treatments, and often indicate the likelihood of benefiting from a specific therapy. For example, molecular biomarkers situated at the interface of pathology-specific molecular process architecture and drug mechanism of action promise capturing aspects allowing assessment of an individual treatment response.[8] This offers a dual approach to both seeing trends in retrospective studies and using biomarkers to predict outcomes. For example, in metastatic colorectal cancer predictive biomarkers can serve as a way of evaluating and improving patient survival rates and in the individual case by case scenario, they can serve as a way of sparing patients from needless toxicity that arises from cancer treatment plans.[9]

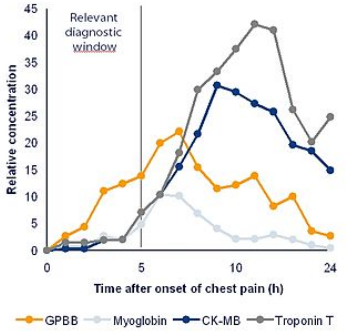
Common examples of predictive biomarkers are genes such as ER, PR and HER2/neu in breast cancer, BCR-ABL fusion protein in chronic myeloid leukaemia, c-KIT mutations in GIST tumours and EGFR1 mutations in NSCLC.[10]

Diagnostic

Diagnostic biomarkers that meet a burden of proof can serve a role in narrowing down diagnosis. This can lead to diagnosis that are significantly more specific to individual patients.

A biomarker can be a traceable substance that is introduced into an organism as a means to examine organ function or other aspects of health.[11] For example, rubidium chloride is used as a radioactive isotope to evaluate perfusion of heart muscle.

It can also be a substance whose detection indicates a particular disease state, for example, the presence of an antibody may indicate an infection.[11] More specifically, a biomarker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment.[11]



After a heart attack a number of different cardiac biomarkers can be measured to determine exactly when an attack occurred and how severe it was.

One example of a commonly used biomarker in medicine is prostate-specific antigen (PSA). This marker can be measured as a proxy of prostate size with rapid changes potentially indicating cancer. The most extreme case would be to detect mutant proteins as cancer specific biomarkers through Selected reaction monitoring (SRM), since mutant proteins can only come from an existing tumor, thus providing ultimately the best specificity for medical purposes.[12]

Another example is KRAS, an oncogene that encodes a GTPase involved in several signal transduction pathways. Biomarkers for precision oncology are typically utilized in the molecular diagnostics of chronic myeloid leukemia, colon, breast, and lung cancer, and in melanoma.[13]

Prognostic

A prognostic biomarker provides information about the patients overall outcome, regardless of therapy.[10]

Research

Biomarkers for precision medicine are a part of a relatively new clinical toolset. In the case of metastatic colorectal cancer (mCRC) only two predictive biomarkers have so far been identified and implemented clinically.[9] In this case, the lack of data beyond retrospective studies and successful biomarker-driven approaches was suggested to be principal cause behind a need for novel biomarker studies within the medical field due to the severe attrition that accompanies clinical trials.

The field of biomarker research is also expanding to include a combinatorial approach to identifying biomarkers from multi-omic sources. Combining groups of biomarkers from various omic data allows for the possibility of developing panels that evaluate treatment response based on many biomarkers at a single time. One such area of expanding research in multi-omic biomarkers is mitochondrial DNA sequencing. Mutations in mitochondrial DNA have been shown to correlate to risk, progression, and treatment response of head and neck squamous cell carcinoma.[14] In this example, a relatively low cost sequencing pipeline was shown to be able to detect low frequency mutations within tumor-associated cells. This highlights the general snapshot capability of mitochondrial DNA-based biomarkers in capturing heterogeneity amongst individuals.[14]

Regulatory validation for clinical use

The Early Detection Research Network (EDNR) compiled a list of seven criteria by which biomarkers can be assessed in order to streamline clinical validation.[15]

Proof of concept

Previously used to identify the specific characteristics of the biomarker, this step is essential for doing an *in situ* validation of these benefits. The biologic rationale of a study must be assessed on a small scale before any large scale studies can occur.[15] Many candidates must be tested to select the most relevant ones.[16]

Experimental validation

This step allows the development of the most adapted protocol for routine use of the biomarker. Simultaneously, it is possible to confirm the relevance of the protocol with various methods (histology, PCR, ELISA, ...) and to define strata based on the results.

Analytical performances validation

One of the most important steps, it serves to identify specific characteristics of the candidate biomarker before developing a routine test.[17] Several parameters are considered including:

- sensitivity
- specificity
- robustness
- accuracy
- reproducibility
- practicality [15]
- ethicality [18]

Protocol standardization

This optimizes the validated protocol for routine use, including analysis of the critical points by scanning the entire procedure to identify and control the potential risks.

Ethical issues

In 1997 the National Institute of Health suggested a need for guidelines and legislation development that would regulate the ethical dimensions of biomarker studies.[15] Similar to the way that the Human Genome Project collaborated with the U.S. Office of Technology Assessment, biomarker susceptibility studies should collaborate to create ethical guidelines that can be implemented into the groundwork and proposal requirements of the studies.

Ensuring that all of the participants that are included each step of the project (i.e. planning, implementation, and the compilation of the results) are provided with the protection of ethical principles that are put in place prior to beginning the project. These ethical protections should not only protect the participants in the study, but also the non participants, researchers, sponsors, regulators, and all other persons or groups involved in the study.[15] Some ethical protections could include but are not limited to:[15]

- Informed consent of the participant
- Access to participation opportunities independent of race, socio-economic status, gender, sexuality, etc. (within the range allowed by the experimental protocol)
- Scientific integrity
- Confidentiality of data (anonymity)
- Acknowledgement of conflict of interest in terms of funding and sponsorship by given sponsors
- Transparency and recognition of health and legal risks involved in participation

Cell biology

In cell biology, a biomarker is a molecule that allows the detection and isolation of a particular cell type (for example, the protein Oct-4 is used as a biomarker to identify embryonic stem cells).[19]

In genetics, a biomarker (identified as genetic marker) is a DNA sequence that causes disease or is associated with susceptibility to disease. They can be used to create genetic maps of whatever organism is being studied.

Applications in chemistry, geology and astrobiology

A biomarker can be any kind of molecule indicating the existence, past or present, of living organisms. In the fields of geology and astrobiology, biomarkers, versus geomarkers, are also known as biosignatures. The term biomarker is also used to describe biological involvement in the generation of petroleum. Biomarkers were used in the geo-chemical investigation of an oil spill in the San Francisco Bay, California in 1988.[20] On April 22–23 around 400,000 gallons of crude oil was accidentally released into the San Joaquin Valley by a refinery and manufacturing complex of the Shell Oil

Company. The oil affected many surrounding areas. Samples of the crude oil were collected in the various regions where it had spread and compared to samples that were unreleased in an attempt to distinguish between the spilled oil and the petrogenic background present in the spill area.[20] Mass Spectra was performed to identify biomarkers and cyclic aliphatic hydrocarbons within the samples. Variations in the concentration of constituents of the crude oil samples and sediments were found.[20]

Ecotoxicology

Rachel Carson, the author of *Silent Spring*, raised the issue of using organochlorine pesticides and discussed the possible negative effects that said pesticides have on living organisms.[21] Her book raised ethical issues against chemical corporations that were controlling the general reception of the effect of pesticides on the environment, which pioneered the need for ecotoxicological studies. Ecotoxicologial studies could be considered the precursors to biomarker studies.[22] Biomarkers are used to indicate an exposure to or the effect of xenobiotics which are present in the environment and in organisms. The biomarker may be an external substance itself (e.g. asbestos particles or NNK from tobacco), or a variant of the external substance processed by the body (a metabolite) that usually can be quantified.

History

The widespread use of the term "biomarker" dates back to as early as 1980.[23] The manner in which the environment was monitored and studied near the end of the 1980s was still mainly reliant on the study of chemical substances that were considered dangerous or toxic when found in moderate concentrations in water, sediments, and aquatic organisms.[22] The methods used to identify these chemical compounds were chromatography, spectrophotometry, electrochemistry, and radiochemistry.[22] Although these methods were successful in elucidating the chemical makeup and concentrations present in the environment of the contaminants and the compounds in question, the tests did not provide data that was informative on the impact of a certain pollutant or chemical on a living organism or ecosystem. It was proposed that characterizing biomarkers could create a warning system to check in on the well being of a population or an ecosystem before a pollutant or compound could wreak havoc on the system. Now, due to the development of biomarker studies, biomarkers can be used and applied in the fields of human medicine and in the detection of diseases.[22]

Definition

The term "biological marker" was introduced in 1950s.[24][25]

- In 1987, biological markers were defined as “indicators signaling events in biological systems or samples” that could be classified into three categories: exposure, effect and susceptibility markers.[26]
- In 1990, McCarthy and Shugart defined biomarkers as, “measurements at the molecular, biochemical, or cellular level in either wild populations from contaminated habitats or in organisms experimentally exposed to pollutants that indicate that the organism has been exposed to toxic chemicals, and the magnitude of the organism's response”.[27]
- In 1994, Depledge defined a biomarker as, “a biochemical, cellular, physiological or behavioral change which can be measured in body tissues or fluids or at the level of the whole organism that reveals the exposure at/or the effects of one or more chemical pollutants.”[28]
- In 1996, Van Gestel and Van Brummelen attempted to redefine biomarkers to unambiguously differentiate a biomarker from a bioindicator. According to Van Gestel and Van Brummelen, a biomarker by definition should be used only to describe sublethal biochemical changes resulting from individual exposure to xenobiotics.[29]
- In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[30][2]
- In 2000, De Lafontaine defined the term biomarker as a “biochemical and/or physiological change(s) in organisms exposed to contaminants, and thus represent initial responses to environmental perturbation and contamination".[31]

Active biomonitoring

De Kock and Kramer developed the concept of active biomonitoring in 1994. Active biomonitoring is a comparison of the chemical/biological properties of a sample that has been relocated to a new environment that contains different conditions than its original environment.[32]

See also

- Bioindicator
- Biomarker discovery
- *Biomarkers* (journal)

- *Biomarker Insights* – a journal
- Imaging biomarker
- Endophenotype
- Molecular marker
- Saliva testing
- Sponge biomarkers

References

1. Hirsch, Michelle S.; Watkins, Jaclyn (May 2020). "A Comprehensive Review of Biomarker Use in the Gynecologic Tract Including Differential Diagnoses and Diagnostic Pitfalls" (https://journals.lww.com/10.1097/PAP.0000000000000238). *Advances in Anatomic Pathology*. **27** (3): 164–192. doi:10.1097/PAP.0000000000000238 (https://doi.org/10.1097%2FPAP.0000000000000238). ISSN 1072-4109 (https://www.worldcat.org/issn/1072-4109).
2. Biomarkers Definitions Working Group (March 2001). "Biomarkers and surrogate endpoints: preferred definitions and conceptual framework". *Clinical Pharmacology and Therapeutics* (Review). **69** (3): 89–95. doi:10.1067/mcp.2001.113989 (https://doi.org/10.1067%2Fmcp.2001.113989). PMID 11240971 (https://pubmed.ncbi.nlm.nih.gov/11240971). S2CID 288484 (https://api.semanticscholar.org/CorpusID:288484). as cited in Siderowf A, Aarsland D, Mollenhauer B, Goldman JG, Ravina B (April 2018). "Biomarkers for cognitive impairment in Lewy body disorders: Status and relevance for clinical trials". *Movement Disorders* (Review). **33** (4): 528–536. doi:10.1002/mds.27355 (https://doi.org/10.1002%2Fmds.27355). PMID 29624752 (https://pubmed.ncbi.nlm.nih.gov/29624752). S2CID 4634411 (https://api.semanticscholar.org/CorpusID:4634411).
3. Babrak LM, Menetski J, Rebhan M, Nisato G, Zinggeler M, Brasier N, et al. (2019). "Traditional and Digital Biomarkers: Two Worlds Apart?" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7015353). *Digital Biomarkers*. **3** (2): 92–102. doi:10.1159/000502000 (https://doi.org/10.1159%2F000502000). PMC 7015353 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7015353). PMID 32095769 (https://pubmed.ncbi.nlm.nih.gov/32095769).
4. Rovini E, Maremmani C, Cavallo F (2017). "How Wearable Sensors Can Support Parkinson's Disease Diagnosis and Treatment: A Systematic Review" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5635326). *Frontiers in Neuroscience*. **11**: 555. doi:10.3389/fnins.2017.00555 (https://doi.org/10.3389%2Ffnins.2017.00555). PMC 5635326 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5635326). PMID 29056899 (https://pubmed.ncbi.nlm.nih.gov/29056899).
5. Brasier N, Raichle CJ, Dörr M, Becke A, Nohturfft V, Weber S, et al. (January 2019). "Detection of atrial fibrillation with a smartphone camera: first prospective, international, two-centre, clinical validation study (DETECT AF PRO)" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6321964). *Europace*. **21** (1): 41–47. doi:10.1093/europace/euy176 (https://doi.org/10.1093%2Feuropace%2Feuy176). PMC 6321964 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6321964). PMID 30085018 (https://pubmed.ncbi.nlm.nih.gov/30085018).
6. Low DM, Bentley KH, Ghosh, SS (January 2020). "Automated assessment of psychiatric disorders using speech: A systematic review" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7042657). *Laryngoscope Investigative Otolaryngology*. **5** (1): 96–116. doi:10.1002/lio2.354 (https://doi.org/10.1002%2Flio2.354). PMC 7042657 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7042657). PMID 32128436 (https://pubmed.ncbi.nlm.nih.gov/32128436).
7. Brasier N, Eckstein J (2019). "Sweat as a Source of Next-Generation Digital Biomarkers" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7011725). *Digital Biomarkers*. **3** (3): 155–165. doi:10.1159/000504387 (https://doi.org/10.1159%2F000504387). PMC 7011725 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7011725). PMID 32095774 (https://pubmed.ncbi.nlm.nih.gov/32095774).
8. Lukas A, Heinzel A, Mayer B (2019-03-11). "Biomarkers for capturing disease pathology as molecular process hyperstructure". bioRxiv 10.1101/573402 (https://doi.org/10.1101%2F573402).
9. Ruiz-Bañobre J, Kandimalla R, Goel A (2019-03-28). "Predictive Biomarkers in Metastatic Colorectal Cancer: A Systematic Review" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7446314). *JCO Precision Oncology*. **3** (3): 1–17. doi:10.1200/PO.18.00260 (https://doi.org/10.1200%2FPO.18.00260). PMC 7446314 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7446314). PMID 32914007 (https://pubmed.ncbi.nlm.nih.gov/32914007).
10. Oldenhuis CN, Oosting SF, Gietema JA, de Vries EG (May 2008). "Prognostic versus predictive value of biomarkers in oncology". *European Journal of Cancer*. **44** (7): 946–53. doi:10.1016/j.ejca.2008.03.006 (https://doi.org/10.1016%2Fj.ejca.2008.03.006). PMID 18396036 (https://pubmed.ncbi.nlm.nih.gov/18396036).
11. "NCI Dictionary of Cancer Terms" (https://www.cancer.gov/publications/dictionaries/cancer-terms). *National Cancer Institute*. 2011-02-02. Retrieved 2020-02-17.
12. Wang Q, Chaerkady R, Wu J, Hwang HJ, Papadopoulos N, Kopelovich L, et al. (February 2011). "Mutant proteins as cancer-specific biomarkers" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3038743). *Proceedings of the National Academy of Sciences of the United States of America*. **108** (6): 2444–9. Bibcode:2011PNAS..108.2444W (https://ui.adsabs.harvard.edu/abs/2011PNAS..108.2444W). doi:10.1073/pnas.1019203108 (https://doi.org/10.1073%2Fpnas.1019203108). PMC 3038743 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3038743). PMID 21248225 (https://pubmed.ncbi.nlm.nih.gov/21248225).
13. Nalejska E, Mączyńska E, Lewandowska MA (June 2014). "Prognostic and predictive biomarkers: tools in personalized oncology" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4031398). *Molecular Diagnosis & Therapy*. **18** (3): 273–84. doi:10.1007/s40291-013-0077-9 (https://doi.org/10.1007%2Fs40291-013-0077-9). PMC 4031398 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4031398). PMID 24385403 (https://pubmed.ncbi.nlm.nih.gov/24385403).

14. Schubert AD, Channah Broner E, Agrawal N, London N, Pearson A, Gupta A, et al. (February 2020). "Somatic mitochondrial mutation discovery using ultra-deep sequencing of the mitochondrial genome reveals spatial tumor heterogeneity in head and neck squamous cell carcinoma" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6980748). *Cancer Letters*. **471**: 49–60. doi:10.1016/j.canlet.2019.12.006 (https://doi.org/10.1016%2Fj.canlet.2019.12.006). PMC 6980748 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6980748). PMID 31830557 (https://pubmed.ncbi.nlm.nih.gov/31830557).
15. *The early detection research network :translational research to identify early cancer and cancer risk : initial report*. Washington, D.C. 2000. hdl:2027/mdp.39015049497442 (https://hdl.handle.net/2027%2Fmdp.39015049497442).
16. "Proof-of-concept study of biomarker development in mice provides a roadmap for a similar approach in humans" (http://www.fredhutch.org/en/news/releases/2011/06/biomarker_development_in_mice.html). *www.fredhutch.org*. 2011-07-19. Retrieved 2015-05-13.
17. Aizpurua-Olaizola O, Toraño JS, Falcon-Perez JM, Williams C, Reichardt N, Boons GJ (March 2018). "Mass spectrometry for glycan biomarker discovery". *Trends in Analytical Chemistry*. **100**: 7–14. doi:10.1016/j.trac.2017.12.015 (https://doi.org/10.1016%2Fj.trac.2017.12.015).
18. "Environmental health perspectives. 105/SUPP.4" (https://babel.hathitrust.org/cgi/pt?id=uc1.31210012258412&view=1up&seq=46). *HathiTrust*. Retrieved 2020-02-17.
19. Biomarkers for Psychiatric Disorders. Publisher: Springer U.S. doi:10.1007/978-0-387-79251-4 (https://doi.org/10.1007%2F978-0-387-79251-4) Copyright: 2009 ISBN 978-0-387-79250-7 (Print) 978-0-387-79251-4 (Online)
20. Hostettler FD (1991). *Geochemical investigation of an oil spill in San Francisco Bay, California*. Menlo Park, CA: US Department of the Interior, Geological Survey. hdl:2027/uc1.31210025025220 (https://hdl.handle.net/2027%2Fuc1.31210025025220).
21. Carson R (2000). *Silent spring*. ISBN 978-0-14-118494-4. OCLC 934630161 (https://www.worldcat.org/oclc/934630161).
22. Amiard-Triquet C, Amiard JC, Rainbow PS, eds. (2016-04-19). *Ecological Biomarkers*. CRC Press. doi:10.1201/b13036 (https://doi.org/10.1201%2Fb13036). ISBN 978-0-429-11149-5.
23. Aronson JK (May 2005). "Biomarkers and surrogate endpoints" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1884846). *British Journal of Clinical Pharmacology*. **59** (5): 491–4. doi:10.1111/j.1365-2125.2005.02435.x (https://doi.org/10.1111%2Fj.1365-2125.2005.02435.x). PMC 1884846 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1884846). PMID 15842546 (https://pubmed.ncbi.nlm.nih.gov/15842546).
24. Porter KA (August 1957). "Effect of homologous bone marrow injections in x-irradiated rabbits" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2082598). *British Journal of Experimental Pathology*. **38** (4): 401–12. PMC 2082598 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2082598). PMID 13460185 (https://pubmed.ncbi.nlm.nih.gov/13460185).
25. Basu PK, Miller I, Ormsby HL (March 1960). "Sex chromatin as a biologic cell marker in the study of the fate of corneal transplants". *American Journal of Ophthalmology*. **49** (3): 513–5. doi:10.1016/0002-9394(60)91653-6 (https://doi.org/10.1016%2F0002-9394%2860%2991653-6). PMID 13797463 (https://pubmed.ncbi.nlm.nih.gov/13797463).
26. "Biological markers in environmental health research. Committee on Biological Markers of the National Research Council" (https://doi.org/10.1289%2Fehp.87743). *Environmental Health Perspectives*. **74**: 3–9. 1987-10-01. doi:10.1289/ehp.87743 (https://doi.org/10.1289%2Fehp.87743). ISSN 0091-6765 (https://www.worldcat.org/issn/0091-6765).
27. McCarthy JF, Shugart LR (2018-01-18). "Biological Markers of Environmental Contamination". *Biomarkers of Environmental Contamination*. CRC Press. pp. 3–14. doi:10.1201/9781351070263-2 (https://doi.org/10.1201%2F9781351070263-2). ISBN 978-1-351-07026-3.
28. Depledge MH (2020-01-29). "The Rational Basis for the Use of Biomarkers as Ecotoxicological Tools". *Nondestructive Biomarkers in Vertebrates*. CRC Press. pp. 271–295. doi:10.1201/9780367813703-20 (https://doi.org/10.1201%2F9780367813703-20). ISBN 978-0-367-81370-3.
29. Van Gestel CA, Van Brummelen TC (August 1996). "Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms". *Ecotoxicology*. **5** (4): 217–25. doi:10.1007/bf00118992 (https://doi.org/10.1007%2Fbf00118992). PMID 24193812 (https://pubmed.ncbi.nlm.nih.gov/24193812). S2CID 195240026 (https://api.semanticscholar.org/CorpusID:195240026).
30. Strimbu K, Tavel JA (November 2010). "What are biomarkers?" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3078627). *Current Opinion in HIV and AIDS*. **5** (6): 463–6. doi:10.1097/COH.0b013e32833ed177 (https://doi.org/10.1097%2FCOH.0b013e32833ed177). PMC 3078627 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3078627). PMID 20978388 (https://pubmed.ncbi.nlm.nih.gov/20978388).
31. Gagné F, Blaise C, Costan G, Gagnon P, Chan HM (August 2000). "Biomarkers in zebra mussels (Dreissena polymorpha) for the assessment and monitoring of water quality of the St Lawrence River (Canada)". *Aquatic Toxicology*. **50** (1–2): 51–71. doi:10.1016/s0166-445x(99)00094-6 (https://doi.org/10.1016%2Fs0166-445x%2899%2900094-6). PMID 10930650 (https://pubmed.ncbi.nlm.nih.gov/10930650).
32. Smolders R, Voets J, Bervoets L, Blust R, Wepener V (2005-07-15). "Active Biomonitoring (ABM) by Translocation of Bivalve Molluscs". *Water Encyclopedia*. John Wiley & Sons, Inc. doi:10.1002/047147844x.wq16 (https://doi.org/10.1002%2F047147844x.wq16). ISBN 0-471-47844-X.
