LABORATORY DIAGNOSTICS OF PARASITIC DISEASES IN POLAND AND ITS COSTS

Diagnostyka laboratoryjna chorób pasożytniczych w Polsce z uwzględnieniem kosztów badań

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ABSTRACT

The paper discusses the problem of parasitic diseases, which is an important public health issue. Laboratory diagnostic methods are described. Sources of financing of laboratory diagnostics of parasitic diseases and its costs for the Polish health care system are also presented.

To confirm a case of parasitic infection and to implement treatment, diagnostic examinations involving microscopic, immunological, and molecular methods are required. In vitro and in vivo cultures are used less frequently.

Microscopic methods are a “gold standard” in the detection of parasites of the intestinal and urinary tracts and the cardiovascular system; they are easy to perform and do not demand high financial outlays. To obtain correct results of microscopic examination an experienced parasitologist and a calibrated microscope are needed. A positive control should always be included in the examined panel of samples.

Serological tests for specific antibodies against parasites are often used by microbiological laboratories. In Poland, such tests are most often employed for the diagnosis of toxoplasmosis, toxocarosis, trichinellosis, alveococcosis, hydatidosis, and seldom for other parasitic diseases (e.g., tropical ones).

Immunological methods for the detection of parasite antigens are more valuable than antibody detection, because positive results point to the actual presence of parasites in the body. To confirm these results microscopic or molecular diagnostic tests ought to be perform. Molecular techniques are used if the parasites are extremely similar or if the infection level is below the microscopic limit of detection. Molecular tests should be performed in reference laboratories with appropriate infrastructure and highly qualified personnel, and for these reasons they are among the most expensive diagnostic tests. Financing parasitological diagnosis by the National Health Fund (NHF) is conducted in different ways, depending on the level of health care provision. At the level of primary health care (PHC), laboratory examinations are financed from capitation fees. Under specialized outpatient care (SOC), until 1 July 2011, the examinations were financed as part of financing medical consultations (e.g., comprehensive, specialized, or diagnostic-curative). Under hospital care, the costs of testing for parasites are included in the reimbursement of hospital stay according to diagnosis related groups (DRG).

The costs of parasitological examinations of the entire health care system are the sum of products of the test cost times the number of tests performed. The data on the number of diagnostic tests performed are incomplete, and the tests prices for parasitic infections may be different in particular diagnostic units. Therefore, it is not possible to conduct a complete economic analysis of parasitological diagnostics.

Keywords: parasites, parasitic diseases, costs of parasitic infection diagnostics, laboratory diagnostics of parasitoses

Słowa kluczowe: pasożyty, choroby pasożytnicze, koszt badań diagnostycznych inwazji pasożytniczych, badania laboratoryjne parazytoz
INTRODUCTION

Parasitic diseases are an important issue for the world’s public health. Even though this problem mostly concerns economically underprivileged countries in the tropical climate zone, some tropical parasitoses may be brought to Poland. Also in our country there occur many species of indigenous human parasites. Even though the Act on the Prevention and Control of Infections and Infectious Diseases in Humans of December 5, 2008 enumerates only 7 parasitic diseases which are mandatory reported (echinococcosis, giardiosis, cryptosporidiosis, malaria, congenital toxoplasmosis, cysticercosis, and trichinellosis), in practice physicians may come across several dozen other types of parasitic invasions. To confirm a case of infection followed by effective treatment, it is necessary to conduct laboratory tests, which involve microscopic, immunological, and molecular methods (in vitro or in vivo cultures are less often used).

Table 1. Laboratory tests for parasitic diseases (parasites) that can be performed in Poland

<table>
<thead>
<tr>
<th>Parasitosis</th>
<th>Parasite</th>
<th>Diagnostic tests</th>
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<td>Immunological: detecting: Anti-bodies</td>
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<td>A1; A2</td>
<td>B1.1</td>
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<td>A1, A4; A6</td>
<td>B1.1</td>
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<td>A1, A6, A7</td>
<td>B1.1</td>
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<td></td>
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<td></td>
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<tr>
<td>Parasite</td>
<td>Species/Genus</td>
<td>A1</td>
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<tr>
<td>Toxocarosis</td>
<td>Toxocara cati, T. canis</td>
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<td>Trichuriasis</td>
<td>Trichuris trichiura</td>
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<tr>
<td>Enterobiasis</td>
<td>Enterobius vermicularis</td>
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<td>Strongyloïdiasis</td>
<td>Strongyloides stercoralis</td>
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<td>A2</td>
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<tr>
<td>Ancylostomias / Necatoriasis</td>
<td>Ancylostoma duodenale, Necator americanus</td>
<td>A1</td>
<td></td>
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<tr>
<td>Trichostrongylidosis</td>
<td>Trichostrongylus spp.</td>
<td>A1</td>
<td></td>
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<tr>
<td>Schistosomiasis</td>
<td>Schistosoma mansoni, S. japonicum, S. haematobium; less often S. intercalatum, S. mathei, S. bovis</td>
<td>A1</td>
<td>A3</td>
</tr>
<tr>
<td>Fascioliasis, Dicrocoeliiasis, Opisthorchiasis</td>
<td>Fasciola hepatica, F. gigantica Dicrocoelium dendriticum, Opisthorchis felineus, O. viverrini</td>
<td>A1</td>
<td>A6</td>
</tr>
<tr>
<td>Trematodiasis</td>
<td>Non-indigenous hermaphroditic trematoda (Fasciolopsis buski, Clonorchis sinensis and others)</td>
<td>A1</td>
<td></td>
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<tr>
<td>Paragonimiasis</td>
<td>Paragonimus westermani and Paragonimus spp.</td>
<td>A1</td>
<td>A4</td>
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<tr>
<td>Malaria</td>
<td>Plasmodium falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi</td>
<td>A5</td>
<td>B1.2</td>
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<tr>
<td>Babesiosis</td>
<td>Babesia divergens, B. microti, B. venatorum</td>
<td>A5</td>
<td>B2</td>
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<tr>
<td>Disease</td>
<td>Organisms</td>
<td>Genes</td>
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<tr>
<td>African trypanosomiasis (sleeping sickness)</td>
<td>Trypanosoma gambiense, T. rhodesiense</td>
<td>A5,</td>
<td>B2</td>
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<tr>
<td>American trypanosomiasis (Chagas disease)</td>
<td>Trypanosoma cruzi</td>
<td>A5,</td>
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<td>Visceral leishmaniasis (kala-azar, Dumdum fever)</td>
<td>Leishmania donovani, L. infantum, L. chagasi</td>
<td>A5,</td>
<td>B2</td>
</tr>
<tr>
<td>Cutaneous leishmaniasis</td>
<td>L. major, L. tropica, L. aethiopica</td>
<td>A6,</td>
<td>A7</td>
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<tr>
<td>Macucutaneous leishmaniasis</td>
<td>L. brasiliensis</td>
<td>A6,</td>
<td>A7</td>
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<tr>
<td>Trichinellosis</td>
<td>Trichinella spiralis, T. britovi</td>
<td>A6,</td>
<td>A7</td>
</tr>
<tr>
<td>Cysticercosis</td>
<td>Taenia solium cysticerci</td>
<td>A6</td>
<td>B2</td>
</tr>
<tr>
<td>Echinococcosis (multilocular echinococcosis, unilocular echinococcosis)</td>
<td>Echinococcus multilocularis, E. granulosus</td>
<td>A6,</td>
<td>A7</td>
</tr>
<tr>
<td>Lymphatic filariases</td>
<td>Wuchereria, Brugia, Mansonella</td>
<td>A5</td>
<td>B1.2</td>
</tr>
<tr>
<td>Cutaneous filariases</td>
<td>Onchocerca volvulus, Dirofilaria repens</td>
<td>A6,</td>
<td>A7</td>
</tr>
</tbody>
</table>

**A – Microscopic examinations of:**
- A1 – stool
- A2 – duodenal contents
- A3 – material from the genitourinary system
- A4 – material from the respiratory tract
- A5 – blood, bone marrow (thin and thick smears)
- A6 – other tissues
- A7 – histopathological

**B – Immunological tests**
- B1 – detecting antigens
- B1.1 – coproantigens (antigens in stool)
- B1.2 – antigens in body fluids (blood, pus, cyst contents)
- B2 – serological, detecting specific antibodies of all types
- C – Molecular tests (PCR and others)
- D – Culture
  - D1 – in vitro
  - D2 – in vivo

**Source:** own work
Proposals for diagnostic standards concerning laboratory procedures in medical parasitology have been previously presented by a team of specialists in “laboratory medical parasitology,” and this paper describes only the general principles of the various types of tests. Table 1 shows a list of tests that may be conducted in Poland in diagnosing a parasitic diseases (a given parasitic species).

**Microscopic examinations**

Microscopic examinations still constitute a “golden standard” for detecting parasites of the gastrointestinal, genitourinary, and circulatory systems. From the point of view of a layperson, such examinations seem to be extremely simple and do not require substantial financial outlays. Indeed, to conduct microscopic examinations of tissues, secretions, or excretions, all one usually needs is a light microscope, slides and cover slips, some test tubes and glass rods, a few not too expensive stains, and reagents for parasite concentration. Taking into consideration only these materials, however, one forgets about many factors that significantly add to the costs, and especially the time put in by specialists. While in Poland microscopic examinations are extremely cheap (they cost several zloty), their results are very often erroneous. A point in case is stool examination, where a stool smear in saline or Lugol’s iodine is prepared, to be subsequently examined under a microscope. Simple concentration methods, such as decantation or flotation in a saturated solution of sodium chloride, are used less often, while such concentration methods as flotation in a zinc sulfate solution (the Faust method) or formalin-ethyl acetate sedimentation, which give the best results, are used only in exceptional situations. Due to the fact that the weights of different developmental stages of parasites differ, one should concurrently use two complementary concentration methods – flotation and sedimentation. *In vitro* cultures are used sporadically.

The most important aspect of obtaining reliable results of parasitological examinations is a specialist-parasitologist who has not only theoretical knowledge of the biology and morphology of parasites, but also extensive practical experience. Furthermore, examinations should be performed using calibrated microscopes, which are equipped with a scale enabling one to measure the size of the examined object. Otherwise, one could easily confuse species such as *Entamoeba histolytica sensu lato* and *Entamoeba hartmanni*. It should also be remembered that detection of parasites takes a long time; for instance, according to the CDC requirements, an examination for the presence of intestinal protozoans should cover at least 200 microscopic fields for slides examined under immersion, while testing for malaria should cover at least 20,000 red blood cells. Examination of such a slide takes about 20 minutes. A control sample should be included in every panel of samples. A parasitological examination report should contain information about: the species and life form of the parasite detected, intensity of infection if necessary (e.g., parasitemia in the case of suspected malaria), any other detected objects (e.g., non-pathogenic parasites, human cells, plant material, or artifacts, which are often mistaken for parasites). The above considerations should be taken into account while calculating the price of an examination by the diagnostic laboratory. However, these factors are often not considered, which gives rise to the problem of overdetection of some parasites, especially those of the gastrointestinal system (e.g., *Giardia intestinalis*). An erroneous microscopic reading leads to a delay in making the correct diagnosis and the implementation of an unnecessary antiparasitic treatment, generating economic and psychological losses. Therefore, in performing microscopic examinations, it is of utmost importance to choose a laboratory with personnel specialized in parasitological diagnostics.

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2. CDC – Centers for Disease Control and Prevention.
SEROLOGICAL TESTS

Serological tests for parasitic diseases are often performed by microbiological laboratories. A number of commercial test kits are available, and the test methods are the same as those for detection of bacterial or viral agents. The cost of the test mostly depends on the price of test kits, which may vary significantly among manufacturers. Another major cost component is the number of examined samples. The costs grow when infection is rare and a single sample is tested using a kit intended for a dozen.

In Poland, serological tests are usually carried out in the case of suspected toxoplasmosis, toxocariasis, hydatidosis, and trichinellosis; less frequently for other parasitoses, e.g., tropical ones (leishmaniasis, amebiasis, filariasis).

An important issue is the interpretation of results, especially in the case of persons arriving from a region endemic for a given parasitosis. One should also remember that a positive result may not necessarily indicate an ongoing infection, but a past one. Serological tests are of little use for diagnosing persons with immunodeficiencies.

IMMUNOLOGICAL TESTS FOR DETECTION OF PARASITE ANTIGENS

Detection of antigens is of greater clinical significance than detection of antibodies, because a positive result indicates the presence of a parasite in the organism. Antigens may be detected in stool (coproantigens) and in body fluids.

In Poland, such tests are mostly used for diagnosing giardiasis, cryptosporidiosis, and malaria.

It should be noted that even if the test is highly specific, many false positive results are obtained in the case of a low prevalence of a given parasitic invasion (e.g., in giardiasis). For example, at a specificity of 95% and a prevalence of 2%, one may obtain 7 positive results out of 100 tests performed, 5 of them being false positives. Based on an erroneous diagnosis, the physician will prescribe an antiparasitic course of treatment and further control tests, causing unnecessary costs. That is why, the positive result of a test for parasite antigens (including all tests for malaria) should always be confirmed by a microscopic examination or a molecular test.

MOLECULAR ASSAYS

Molecular techniques are used for diagnostics of parasitic diseases in cases where microscopic identification is ambiguous due to the parasite being very similar to other species (e.g., Entamoeba histolytica–Entamoeba dispar or Cryptosporidium, microsporidia) or due to the invasion level being too low. Such tests are now performed in economically developed countries in reference centers. In Poland, routine molecular tests for parasitoses are performed mostly at the National Institute of Public Health–National Institute of Hygiene (NIZP-PZH) in Warsaw and at the Institute of Maritime and Tropical Medicine, Medical University of Gdańsk. The available tests include tests for congenital toxoplasmosis and neurotoxoplasmosis, molecular diagnostics of malaria with differentiation between Plasmodium species and determination of the drug resistance of the parasite (e.g., Plasmodium falciparum)

as well as tests for amoebiasis,\textsuperscript{9} babesiosis, leishmaniasis, cryptosporidiosis,\textsuperscript{10} acanthamoebiasis,\textsuperscript{11} and diagnostics differentiating \textit{Echinococcus} species.\textsuperscript{12} One can also perform diagnostics to differentiate the various species (genotypes) of \textit{Trichinella} roundworm larvae, which are the etiological factors in trichinellosis; such tests are offered by the NIZP-PZH,\textsuperscript{13} the Institute of Parasitology, Polish Academy of Sciences, and the Institute of Medical Biology and Parasitology, Medical University of Poznań. Since recently, the NIZP-PZH has been determining species of roundworms of the genus \textit{Dirofilaria}, which are spread by mosquitoes in Poland.\textsuperscript{14} Molecular assays should be performed in reference laboratories which have adequate infrastructure, cutting-edge equipment, and highly trained personnel, due to which such tests are some of the most expensive diagnostic procedures.

**Financing of parasitological tests**

The price of tests for parasitic invasions may vary among diagnostic laboratories due to the different prices of reagents, scope of methods employed, personnel costs, indirect costs, and the number of tests performed at the same time. The last item is of great importance in the case of immunological and molecular assays.

Parasitological tests may be ordered privately or by physicians who have contracts with the National Health Fund (NFZ). Such contracts may be concluded at three levels of health care: primary health care (PHC), specialized outpatient care (SOC), or hospital care. The Act on Publicly Funded Health Care Benefits of August 27, 2004 and related regulations define the so-called package of diagnostic procedures financed by the NFZ.

The Regulation of the Minister of Health amending the regulation on guaranteed benefits in primary care of October 22, 2010 defines medical laboratory services, including parasitological tests, in relationship to the services offered by primary care physicians. This regulation, apart from diagnostic tests conducted according to need, includes only stool testing for parasites. Furthermore, the Regulation of the Minister of Health on guaranteed services in specialized outpatient care of May 27, 2011 specifies a list of guaranteed services consisting both of specialist consultations and diagnostic procedures, including laboratory tests. Guaranteed benefits include, among others, twenty eight of the most important parasitological tests, and microscopic parasitological examinations (classified as examinations of biological material). Currently, this regulation is to be amended.

Parasitological testing is financed in different ways, depending on the level of health care at which the services are provided. At the level of the entire health care system, the costs of such tests are covered by the NFZ, but the distribution of funds varies. At the level of primary health care, diagnostic procedures are financed from capitation fees for patients. Under specialized outpatient care (SOC), until 1 July 2011, the tests were financed as part of financing medical consultations (e.g., comprehensive, specialized, or diagnostic-curative). Under hospital care, the costs of testing for parasites are included in the reimbursement of hospital stay according to diagnosis related groups (DRG).

The Regulation of the Minister of Health on general terms and conditions of contracts for the provision of health care benefits of May 6, 2008 clearly states that it is the health care provider that must ensure comprehensive services,
including appropriate diagnostic procedures, such as laboratory tests. However, analyzing the existing system of financing parasitological tests, especially under PHC and SOC, it becomes obvious that currently there is no motivation for ordering more of these tests. One could even argue that the present system encourages physicians to order a minimal number of parasitological tests. As of July 2011, a new system of financing SOC is to be introduced, based on the DRG model used in hospitals. In simple terms, financing will depend on the number of diagnostic procedures performed. In this way, the NFZ wants to reduce the amount of costs of outpatient health care that are being transferred to hospital care and private practices, and to motivate specialist physicians to prescribe complex diagnostic procedures.

**Parasitological tests – costs for the health care system**

Given the limited financial, human, and equipment resources in health care, it is necessary to conduct economic analysis to determine the efficiency, effectiveness, and efficacy of parasitological tests. For a complete economic analysis, one needs a considerable amount of data, and especially data concerning alternative procedures, costs, and consequences of choosing an alternative. In this case, the alternatives are clearly defined: performance or non-performance of a particular parasitological test. Cost data and consequences of choosing an alternative present far greater difficulties.

The costs of parasitological tests for the entire system are the sum of products of the test cost times the number of tests performed. As mentioned above, tests are not financed independently, but within outpatient consultations or hospital stay. Therefore, the NFZ receives reports concerning the number of consultations given, but not the number of diagnostic procedures performed. Furthermore, the prices of diagnostic procedures are not arbitrarily set by the NFZ. Most health care facilities order diagnostic tests from external laboratories, and they negotiate the prices of tests with those laboratories. Therefore, the prices of particular tests may vary greatly, depending on the laboratory and contract. Moreover, large diagnostic laboratories offer parasitological microscopic examinations below their actual costs, and make up for the losses with other tests performed on a mass scale.

Due to the lack of elementary data, it is impossible to conduct a complete economic analysis of parasitological testing. However, one may attempt an incomplete economic analysis of a given type of test, e.g., for congenital toxoplasmosis.

Toxoplasmosis is caused by *Toxoplasma gondii* protozoan and is probably the most widespread parasitic infection in Poland. A seroepidemiological study conducted in the Poznań region revealed toxoplasmosis infection in 81.8% of women aged 40-45. In immunologically competent persons, toxoplasmosis does not present a serious threat to health, and in most cases such an infection is asymptomatic. However, once acquired, the infection remains in a latent form throughout the life of the infected person and may be activated in the case of an abrupt loss of immunity, e.g., due to cancer treatment, immunological disease, or immunosuppression linked to an organ transplant or HIV infection (in AIDS patients). Primary infection with *T. gondii* in pregnant women may lead to the parasite being transmitted to the fetus, in which it causes serious damage to the central nervous system, eyes, and other internal organs. Congenital toxoplasmosis is a very frequent condition in Poland: another study carried out in the Poznań region showed that 1 in 1000 live born children is infected with *T. gondii*. Due to the fact that in the years 2009-2010 only 9 cases of congenital toxoplasmosis were reported to the epidemiological supervision authority, one may assume that most cases remain undiagnosed. If a diagnosis is not made in the perinatal period, the infection develops and causes irreversible damage leading to mental retardation, loss of vision, and impaired hearing.

The Regulation of the Minister of Health on standards and medical procedures for providing perinatal care services for women during physiological pregnancy, physiological birth, postpartum and for the newborn of September 2010 specifies that tests for the presence of IgM and IgG antibodies against toxoplasmosis should be performed twice: first, up to the 10th week of pregnancy, and, second, for women which previously did not reveal antibodies, in the 21st-26th week of pregnancy. In previous years, tests were prescribed for serologically negative women in each trimester of pregnancy. Thus, it may be assumed that every pregnant woman has a test for toxoplasmosis. Another 10% should be added to this figure, that is, the number of women who are planning to conceive a child and order this test for preventive purposes. Furthermore, some women repeat the test following a positive result. In 2009, 419,000 children were born in Poland, so the number of tested patients may be estimated at about 460,000. The market price of an IgM and IgG test is in the range of PLN 20-40. The price of testing for congenital toxoplasmosis is much higher, ranging from PLN 130 to 500. Taking into account only the costs of the first test, the total expenditure is estimated at PLN 9.2-18.4 million. Last but not least, it should be noted that the above figure is bound to be considerably overestimated because health care facilities negotiate lower prices while signing contracts with laboratories.

These costs are then compared to opportunity cost, that is, non-performance of the test. As it was mentioned above, failure to diagnose a disease results in irreversible changes leading to a number of serious disorders. Under the circumstances, it is difficult to estimate how many cases of the kind occur in Poland. Taking into consideration only some components of social costs, e.g., costs of unemployment, costs of medical and long-term care, costs of lost income of ill or disabled persons and their caregivers, and costs borne by the state (disability pensions, etc.), one may assume that the total costs are extremely high. Studies conducted in various countries report very high additional costs linked to, e.g., disability and other health problems. They mention costs of lost income, additional costs, costs of additional resources (e.g., equipment) and the related lower living standards, alternative costs (e.g., extra time for performing activities of daily living).

**Summary**

In summary, analysis of the cost effectiveness of tests for congenital toxoplasmosis may only be conducted partially, because we do not know the total number of cases of congenital toxoplasmosis or how many disorders are due to undiagnosed toxoplasmosis. It is not possible to precisely determine the efficiency of parasitological tests. Based on preliminary analysis and other cost effectiveness studies, one may assume that the benefits from performing a greater number of parasitological tests will greatly outweigh their costs. The principal benefit of such tests is prevention of future complications, thus avoiding considerable social costs.

Moreover, due to the special model of financing of parasitological tests, one may expect that the actual number infections is considerably underestimated, which generates additional costs for the health care system. A good solution for reducing future costs would be the introduction of screening of newborns for toxoplasmosis, which would make it possible to identify all cases of infection.

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REFERENCES


