MINISTERIO
DE SANIDAD
Y POLÍTICA SOCIAL

SECRETARIAT-GENERAL FOR HEALTH

DIRECTORATE-GENERAL FOR PUBLIC HEALTH AND FOREIGN HEALTHCARE

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and BLOOD DONATION

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CHAGAS DISEASE AND BLOOD DONATION

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1. CHAGAS DISEASE IN NON-ENDEMIC COUNTRIES

Introduction

American trypanosomiasis or Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is an important public health problem in Latin American countries. It currently affects 16-20 million people. About 100 million are estimated to be exposed to the risk of infection, and approximately 15,000 die each year from this cause.

Traditionally, the disease has been associated with people from impoverished rural areas; however migration has produced substantial changes, and Chagas disease is now a condition diagnosed in the large urban centers of the Americas, as well as in countries in other continents.

Despite an uneven development, programs aimed at controlling the disease in endemic countries have significantly reduced the rate of infection by vectorial transmission (*Dias, 2005*)[1] (*Moncayo, 2003*)[2]. Oral transmission from the intake of contaminated food is associated with the presence of the triatomine vector in endemic areas.

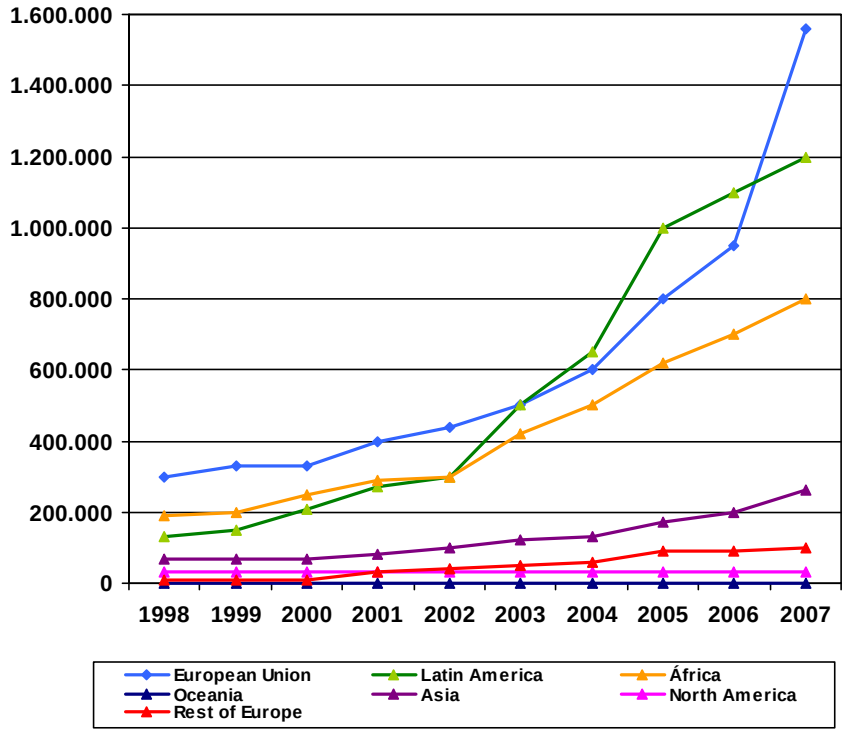
Triatoma infestans

In rural areas of Latin America, vectorial transmission accounts for 80% of the routes of transmission (*Paricio et al., 2008*)[3]. The persistence of the infection and the long asymptomatic period in most infected individuals, together with the large number of people emigrating from endemic zones, make non-vectorial transmission possible in non-endemic areas.

In addition to the vectorial route, *T. cruzi* can be transmitted through the transfusion of blood and blood components, through the transplantation of solid organs donated by infected individuals, and by vertical (congenital) transmission. Other possible routes are oral (breast milk) (*FDA, 2007*)[4] and conjunctivitis. Some cases of transmission caused by laboratory accidents in departments working with the parasite have also been described.

Latin American immigration in Europe and Spain.

For many years, Europe has received immigrants from practically every place in the world; Spain has also become an immigrant host country since the 1980s. This phenomenon has been developing to this day, and in late 2007, immigrants represented 8.79% of the population, growing at a rate of 1.92 percentage points with respect to the previous year. Specifically, Latin American immigration reached 1,594,338 people, of whom approximately 700,000 are women with childbearing potential (*INE, 2008*)[5]. This is the OECD country with the largest number of counted Latin American immigrants; the figures increase significantly as of the year 2000, when they surpassed those of other collectives (Graph 1) (*Observatorio Permanente de la Inmigración, 2007)*[6].



Graph 1:
Registered or valid residence-card bearing foreigners according to continent 1998-2007

Table 1 shows more clearly the magnitude of the migration phenomenon. (INE 2008)[5.]

Table 1.. Evolution of the foreign population from 2000 to 2007.

YEAR	2000	2001	2002	2003	2004	2005	2006	2007
Total population	**40,499,790**	**41,116,842**	**41,837,894**	**42,717,064**	**43,197,684**	**44,108,530**	**44,708,964**	**45,200,737**
Americas	202,440	442,143	754,200	1,081,619	1,276,101	1,488,680	1,557,604	1,638,694
Africans	207,437	298,901	399,836	492,951	541,518	663,156	725,960	737,400
Asians	51,838	70,475	93,329	122,208	135,108	176,290	206,476	207,850
Europeans	460,906	557,600	728,746	965,217	1,079,555	1,400,057	1,651,571	1,932,998
Oceania	1,258	1,540	1,836	2,173	2,044	2,427	2,555	2,612
Total foreigners	**923,879**	**1,370,657**	**1,977,946**	**2,664,168**	**3,034,326**	**3,730,610**	**4,144,166**	**4,519,554**

Of the 4,500,000 foreigners residing in Spain in 2007, 36% came from the Americas.

Chagas disease in Spain.

It is known that a percentage of immigrants living in our country have Chagas disease in a chronic form (indeterminate, chronic cardiac, or chronic digestive) (*Muñoz et al., 2009)*[7].

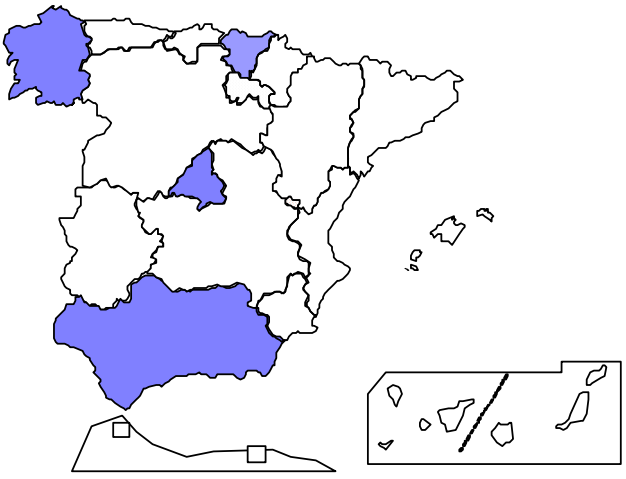
Significantly, in a study conducted in two Barcelona centers specializing in imported diseases, *T. cruzi* was detected in 41% of Latin American adults tested (*Muñoz et al., 2009*)[8], In another study, the prevalence of infection among Latin American pregnant women reached 3.4%, with a rate of vertical transmission of 7.3% (*Muñoz et al., 2007*)[9]. This series showed that the percentage of Bolivian women infected with *T. cruzi* is 27%. Another study, conducted in three maternity centers in the Valencia Community, detected 4.64% of women infected (*Paricio et al., 2008*)[3]. Finally, three cases of vertical transmission have already been published in Spain (*Muñoz et al., 2007*)[9] (*Riera et al., 2006*)[10] *(Flores-Chávez et al., 2008)*[11]

The first known case of transmission through transfusion occurred in 1984, after a bone marrow transplantation; it was published in 1992 (*Villalba et al., 1992)*[12] . However, five cases of Chagas disease secondary to transfusion have been reported since (*Forés et al., 2007)*[13] *(Pérez de Pedro et al., 2008)*[14] *(PEHV)*[15]

Table 2 shows the cases of Chagas disease reported through the State Hemovigilance Program (PEHV)[15], the year when the transmission occurred, and the geographic location represented on Graph 2.

Table 2: Cases of Chagas disease reported through PEHV

		YEAR DETECTED/REPORTED		
		2005	2006	2007
Year of transmission Año de transmisión	**1995**	-	1	-
	2004	-	-	1
	2005	1	-	-
	2006	-	-	1
	2007	-	-	1
Total		**1**	**1**	**3**



Graph 2: Geographic location of reported cases

2. CHAGAS DISEASE AND TRANSFUSION

Trypanosoma cruzi was discovered in 1908; 28 years later, in 1936 in Argentina, Mazza suggested that the disease could be transmitted through blood transfusion (*Mazza et al., 1936)*[16]. Thirteen years later, in 1949 in Brazil, the first *T. cruzi*-infected blood donors were described *(Pellegrino 1949)*[17]. . In 1952, Freitas reported the first cases of Chagas disease from transfusion (*Freitas et al.,* 1952)[18].

History

Brief chronology

1907

- Chagas discovered the parasite in the vectors.

1908

- Chagas found the same parasite in a sick cat.
- Two weeks after finding the parasite in the cat, he found it in the blood of a sick girl with fever.

1909

- Chagas first described the disease.

1911

- Chagas described the first congenital case.

1916

- Chagas suggested the possibility of involvement of the digestive tract.

1936

- In Argentina, Mazza first suggested transmission through transfusion. Subsequently, other authors in Argentina, Brazil, and Uruguay supported this theory.

1949

- Infected blood donors first detected in Brazil.

1951

- Other authors found the same.

1952

- The first two cases of post-transfusion Chagas disease are published.

Transfusional Chagas disease

During the chronic phase, parasitemia is low and intermittent, so a transfusion from a donor with Chagas disease may not be infectious. Transfusion is the second most common cause of transmission of the disease after vectorial transmission, and is more frequent than vertical transmission (*Schumunis, 1999)*[19] *(Barcán et al., 2005)*[20].

The number of post-transfusional cases of Chagas disease has been estimated between 300 (*Wendel, 1998)*[21] and 800 (*Hernández-Becerril et al., 2005)*,[22] however, these figures are considered an underestimate. One of the causes of this underestimation is the existence of asymptomatic individuals infected through transfusion (*Leiby et al., 1999)*[23] *(Pérez de Pedro et al., 2008)*[14.] Transfusion has become an important source of transmission in Latin America, mostly due to migration from rural to urban areas (*Schumunis, 1991)*[24].

In the USA, a seroprevalence if 0.12 – 0.20% has been found among donors of risk, and seven cases of post-transfusional Chagas disease have been published (*Bihl et al., 2007)*[25]. It is possible—even common—that some cases of post-transfusional Chagas disease are not diagnosed; this may be attributable to two reasons:s:

1. Patients are asymptomatic or have very mild manifestations of the disease.
2. Patients die from the condition that motivated the transfusion, leaving no time to make the diagnosis of Chagas disease.

Other possibilities are: 1) individuals received a transfusion years ago and are asymptomatic, but experience a reactivation due to severe immunosuppression, and 2) donors whose mothers had Chagas disease, born outside of, or never having visited, endemic areastras.

Clinical manifestations

Parasitemia in asymptomatic donors is low and intermittent, so a transfusion may not transmit the disease if at the time of donation there are no parasites in blood. In post-transfusional Chagas disease, the incubation period is 20-40 days (range: 8-120 days), which is longer than for vectorial transmission (7-10 days). This has been attributed to the lower infectious capacity of the circulating trypomastigotes compared to that of the metacyclic trypomastigotes excreted by the vector. In endemic areas, 20% of recipients infected through transfusion are totally asymptomatic, which leads to not suspecting the diagnosis (*Wendel, 1998)*[21]

Among the acute symptoms, the most common, and sometimes the only one, is fever. There may be lymphadenopathy and hepatosplenomegaly. Diffuse myocarditis, often accompanied by pericarditis, and meningoencephalitis are the most severe complications of the acute.

Spontaneous recovery after the acute phase occurs in 6-8 weeks, with a maximum of 4 months. In most cases, the disease progresses normally to an indeterminate chronic phase that may last several decades. Later, a chronic phase with heart or gastrointestinal manifestations may develop.

Infection through transfusion

The infectious capacity of one unit of whole blood is 12-25%, with a maximum value of 46.7% in Bolivia (*Wendel,* 2006)[26] Infection through blood and blood products depends on several **factors**:

1. **Type and quantity of the component transfused.**

   The parasite must remain viable throughout processing and manipulation; this is a relatively fragile parasite which may be transmitted through whole blood, packed red blood cells, platelets, and white blood cells.

   Whole blood and platelets seem to be the components with the highest risk of transmission. Since whole blood is rarely used today, the component with the highest risk are platelets. Most published cases in non-endemic areas were caused by this component. The fact that the units of platelets are kept at 20-24°C, a temperature similar to that used for parasite culture, may explain why *T. cruzi* remains viable during the entire preservation period of this blood component (up to 7 days).

   There have been reports that the parasite could live for 2-3 weeks at refrigeration and freezing temperatures, but a longer survival is unknown. Some authors believe that the parasite cannot resist freezing because *T. cruzi* is a cell surrounded by a cell membrane, for which reason ice crystals that develop during freezing can destroy it, as they do when red blood cells are frozen. Adding a cryoprotective agent (glycerol) significantly improves the viability of frozen red blood cells, and the same may happen in the case of *T. cruzi.*

   Other kinds of manipulation such as radiation do not inactivate the parasite; leukocyte reduction, while reducing the number of parasites, does not prevent transmission completely.

Transmission through products obtained from plasma fractionation is unknown.

2. **The parasite itself, depending on the genotype transfused.**

3. **Presence of parasitemia at the time of donation.**

   In order for the disease to be transmitted, the donor must have parasitemia at the time of donation; in most cases, the levels of parasitemia are low. Trypanosomas are parasites with a mostly intracellular tropism, and usually do not circulate freely in the bloodstream.

4. **The immune condition of the recipient.**

   Acute infections are usually detected in immunosuppressed patients. This means that most patients who receive a transfusion are not recognized as infected, even if they actually are. Transmission of *T. cruzi* may occur in immunocompetent patients, but is not detected due to the mildness, or even the asymptomatic nature of some of the forms of the disease. In contrast, in immunosuppressed patients the infection may be severe, and even fatal.

5. **Whether or not screening tests are done.**

   These tests are usually based on the measurement of specific antibodies against *T. cruzi* antigens. These antigens appear on the second week after infection, and do not reach maximum levels until after the third or fourth week.

Description of the cases discussed in the Bibliography

1. USA, 1989º (*Grant et al.,)*[27] An 11-year-old boy with Hodgkin's disease received a **PLATELET** transfusion form a donor from **BOLIVIA** who had emigrated to the USA 15 years before. The patient presented with fever, myopericarditis, and possible meningoencephalitis. Incubation period: **37-67 days**.

2. Canadá, 1989º (*Nickerson et al.,)*[28]. A 21-year-old female living in Canada, with acute lymphoblastic leukemia and protein S deficiency. She had received a **PLATELET** transfusion from a **donor** from **PARAGUAY** who had **emigrated** to the USA **20 years** before. The patient presented with fever and heart failure. **Incubation period**: **60 days**.

3. USA, 1999º *(Leiby et al.,)*[23] A 60-year-old woman with multiple myeloma was found in a review of patients for a study. She had received a **PLATELET** transfusion form a **donor** from **CHILE** who had **emigrated** to the USA **33 years** before. The recipient had no symptoms of Chagas disease, but was positive for parasitemia some 40 days before seroconversion, which occurred approximately 100 days after the transfusion.

4. USA, 2007º *(Young et al.,)*[29]. A 3½-year-old girl with a neuroblastoma received a **PLATELET** transfusion form a **donor** from **BOLIVIA** who had **emigrated** to the USA **17 years** before. The patient presented with fever, neutropenia, and skin manifestations. **Incubation period**: **6 weeks**.

5. Spain, 2008º *(Pérez de Pedro et al.,)*[14] A 33-year-old Spanish male with bone marrow aplasia had received a **PLATELET** transfusion form a **donor** from **BOLIVIA** who had **emigrated** to Spain **3 years** before. The patient presented with fever and skin manifestations. **Incubation period**: **3 months**.

6. Spain, 2008º *(Pérez de Pedro et al.,).*[14] A 57-year-old Moroccan woman operated for choroid plexus papilloma had received a **PLATELET** transfusion from the **same donor as the case above**. The patient was asymptomatic, and the infection was discovered in an epidemiological study; she had positive serology and PCR. Seven units of red blood cells, 5 units of platelets, and 1 unit of plasma had been transfused from the donor. Serology and PCR were tested in the 8 living recipients, and only one case of infection was found (mentioned above). Chagas disease does not seem to be responsible for the death of the five deceased patients who had received a transfusion.

7. Spain, 2008º *(Flores-Chávez, et al.,)*[30]. A 25-year-old Spanish patient with leukemia and bone marrow transplantation had received a **PLATELET** transfusion form a **donor** from **BRAZIL** who had **emigrated** to Spain **1 year** before. The patient presented with fever and multiple organ dysfunction syndrome with CNS involvement. **Incubation period: 48 months**. The patient died despite treatment with benznidazole.

8. Spain, 1992º *(Villalba et al.,)*[12]. A 20-year-old Spanish patient with acute lymphoblastic leukemia who had received two bone marrow transplantations and multiple transfusions (a total of 20 blood products of various types). The donor or the incubation period could not be determined. The patient presented with fever and pericarditis. Treatment with nifurtimox was initiated, but the patient died from septic shock.

3. DETECTION/CONFIRMATION TESTING FOR *TRYPANOSOMA CRUZI* INFECTION

Parasite detection methods have a low sensitivity during the chronic phase; the most sensitive test is DNA detection by PCR. Parasitemia is usually low, intermittent, or absent; for this reason, diagnosis of the infection is based fundamentally on the determination of specific antibodies against *T. cruzi*. It is precisely these antibodies that are responsible for the modulation of parasitemia during the acute phase. In the initial phase, the antibodies are of the IgM type, and are gradually replaced by IgG antibodies. Specific IgG antibodies reach a maximum level after the third or fourth week, and stay elevated if no treatment is received *(Pinto Dias, 2004)* [31]

Serologic diagnostic techniques can be divided in two large groups: The so-called conventional techniques, which use the whole parasite as the antigen, such as indirect immunofluorescence (IIF), or soluble and/or purified extracts containing a complex mixture of antigens such as in indirect hemagglutination (IHA), and enzyme immunoassays (ELISA). In contrast, non-conventional tests use recombinant antigens or synthetic peptides in the ELISA format, particle gel agglutination, immunochromatography, or Western blot *(OMS, 2003)*[32]

Despite new technological advances, no serologic assay has a 100% sensitivity and specificity; thus, confirmatory diagnosis is based on the concordance of at least two techniques with different principles and antigens. When results do not agree, a third assay is indicated *(OMS, 2003)*[32] Additionally, there must be differential diagnosis testing for other infections or diseases that can cause false positive reactions. This is the case for mucocutaneous and visceral leishmaniasis, malaria, sleeping sickness, syphilis, toxoplasmosis, hepatitis, systemic lupus erythematosus, schistosomiasis, rheumatoid arthritis, paracoccidiomycosis, mononucleosis, and autoimmune diseases *(Wendel, 2006)*[26]

For serologic screening used for transfusion, the expert committee of the WHO recommends the use of a conventional test, preferably an ELISA assay, thus sacrificing specificity for a higher sensitivity.

There is no consensus regarding a reference technique. Some authors suggest that the Western blot technique using excreted-secreted antigens and radioimmunoprecipitation of 72 and 90 kDa-glycoproteins (RIPA) may constitute confirmation techniques *(Umezawa et al., 1996)*[33] *(Winkler et al., 1995)*[34]. However, these tests are done only in specialized centers with an infrastructure that allows for maintaining cultures of the infectious forms and radioactive manipulation (I^{125}). In any case, it is noted that the mixture of excreted-secreted antigens (TESA blot) can vary between lots, manufacture limits its profitability, and its use does not eliminate the possibility of a cross-reaction with leishmaniasis *(Amato Neto et al., 2005)*[35]. These disadvantages limit its use to a small number of samples. RIPA is a confirmation technique used mainly in the USA; recent studies suggest that it can also yield false negatives *(Chang et al., 2006)*[36] *(Wendel, 2006)*[26]

Commercial kits based on both total antigens and recombinant antigens are currently available in Spain. Appendix Tables I and II show the technical characteristics of the techniques currently available in the market.

It is difficult to evaluate rigorously which test is most efficient. Performance depends on multiple factors, for which reason continuous evaluation of the reactants is necessary. However, when selecting one technique over another, the objectives and the available infrastructure must be taken into consideration. For **serologic screening**, the most sensitive test should be chosen, usually of the ELISA format, even though an ideal strategy may be to use conventional ELISA combined with a non-conventional technique providing more specificity, thus minimizing the number of unnecessary exclusions. In contrast, for **diagnosis**, either a combination of two conventional tests, or a combination with one of the more specific non-conventional tests should be used. Most non-conventional tests available commercially in Spain are based on very similar antigenic epitopes (Appendix Table I), for which reason the use of two of these reagents would not meet the recommendation to use tests with different principles for confirmation.

Most ELISA tests (Ortho® Clinical Diagnostics, Certest/Abbot Laboratories, BiosChile, Bioelisa Biokit), as well as "in house" ELISA techniques, have a sensitivity of 100%, except for BLK *T. cruzi* IgG ELISA (97.6%) *(Flores-Chávez et al., 2008)*[37]

Conventional techniques have a specificity of 100%; this is not so for tests with recombinant antigens, which may yield false positive results. However, these tests have a specificity of 98 to 99% *(Flores-Chávez et al., 2008)*[37]. The Bioelisa Chagas Biokit, with a 99% specificity uses a conjugate containing anti-IgM in addition to anti-IgG. Although some authors advocate for the diagnostic utility of assessing IgM antibodies *(Betonico et al., 1999)*[38] *(Corral et al., 1998)*[39], these antibodies can yield different *(Boes, 2000)*[40].

One of the main disadvantages is the cross-reactivity with leishmaniasis and malaria. While conventional tests cross-react, especially in patients with leishmaniasis, non-conventional techniques have cross-reaction in patients with malaria *(Flores-Chávez et al., 2008)*[37]

ELISA Ortho® Clinical Diagnostics, Certest/Abbot Laboratories, BiosChile, and Bioelisa Biokit tests currently meet the expectations of serological screening of infection with *T. cruzi*.

4. INCIDENCE OF *TRYPANOSOMA CRUZI* IN BLOOD DONATIONS IN SPAIN.

Blood supply safety policies are different in endemic and non-endemic countries. In the former, donations are tested for anti-*Trypanosoma cruzi* antibodies. In non-endemic countries there are two kinds of approach: Excluding donors who have or have had the disease or who come from risk areas, and accepting donations only if a negative result is obtained in a validated test for anti-*Trypanosoma cruzi* antibodies. This is the case of countries with a large Latin American population such as the USA *(Assal & Aznar., 2007)*[41] *(Stramer et al., 2007)*[42]

In Spain, Latin American immigrants are the potential carriers of Chagas disease, and amount to 1,638,694 in the country. The most common countries of origin are: Ecuador (25%), Colombia (16%), Bolivia (14%), Argentina (8%), Peru (7%), and Brazil (6.6%). The distribution on the territory is not homogeneous (Appendix Table III) *(INE 2008)*[5] Cataluña, Madrid, and Valencia are the three Autonomous Communities with the highest proportion of Latin Americans.

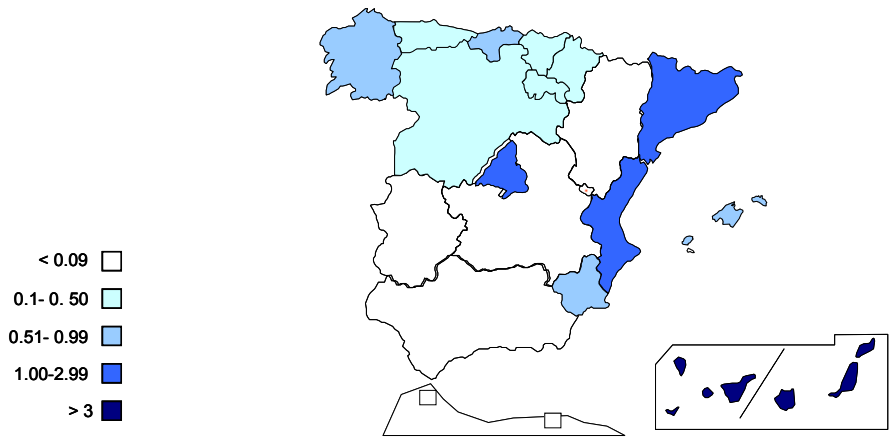
Tables 4 and 5 and the subsequent graphs show the evolution of the screening techniques in blood donations in the various Autonomous Communities.

ANTI-*TRYPANOSOMA CRUZI* SCREENING TESTS IN BLOOD DONATIONS: EVOLUTION

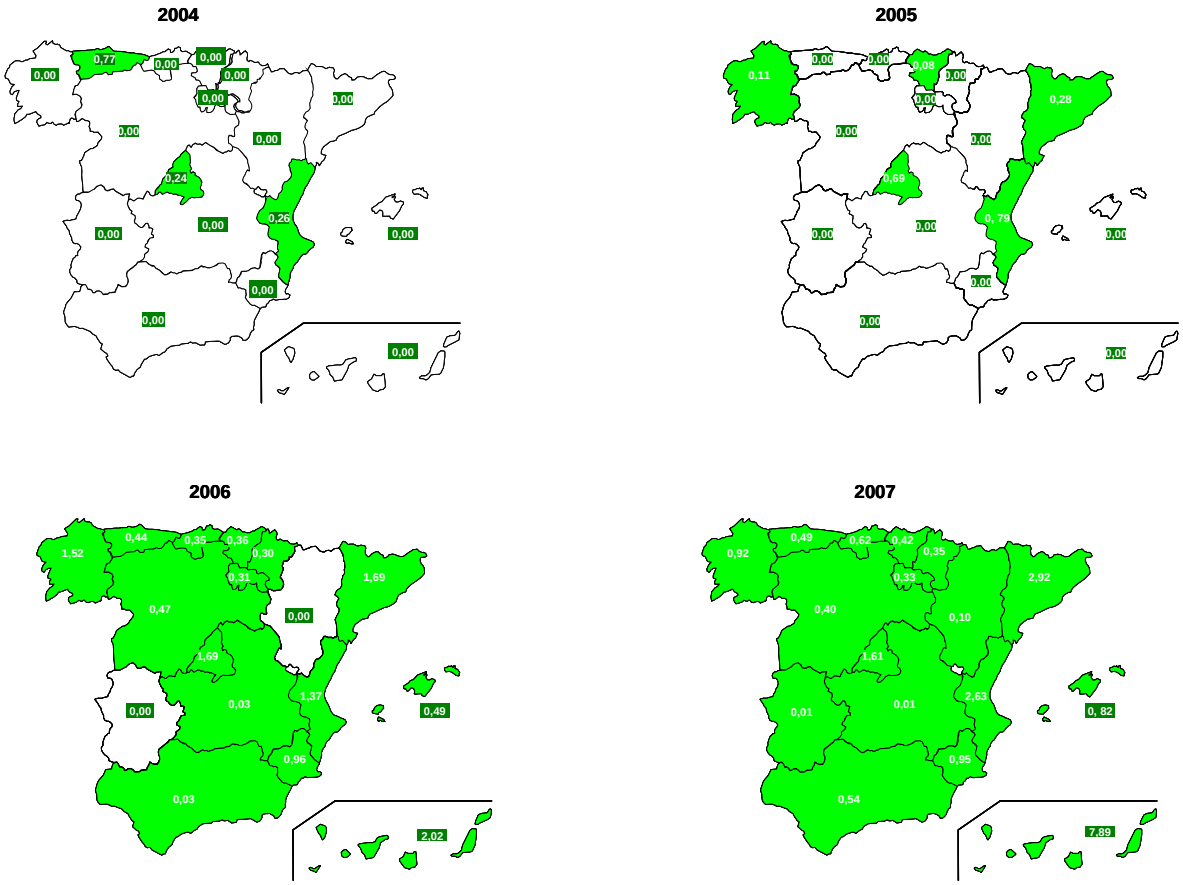
Table 4.

	2004		2005		2006		2007	
AUTONOMOUS COMMUNITIES	**Total Donations**	**% Determ. Total Donations**	**Total Donations**	**% Determ. Total Donations**	**Total Donations**	**% Determ. Total Donations**	**Total Donations**	**% Determ. Total Donations**
ANDALUCÍA	257,798	0.00	259,636	0.00	266,347	0.03	265,533	0.54
ARAGÓN	40,761	0.00	39,431	0.00	40,780	0.00	42,832	0.10
ASTURIAS	39,693	0.77	42,490	0.00	41,887	0.44	41,296	0.49
BALEARES	39,618	0.00	40,809	0.00	39,452	0.49	38,580	0.82
CANARIAS	60,293	0.00	60,852	0.00	60,694	2.02	61,864	7.89
CANTABRIA	23,637	0.00	23,180	0.00	23,223	0.35	23,900	0.62
C.MANCHA	69,535	0.00	67,413	0.00	67,546	0.03	69,059	0.01
C.LEON	86,804	0.00	83,070	0.00	93,311	0.47	95,830	0.40
CATALUÑA	241,314	0.00	249,529	0.28	275,946	1.69	280,434	2.92
EXTREMADURA	43,236	0.00	45,677	0.00	47,187	0.00	45,871	0.01
GALICIA	123,886	0.00	119,109	0.11	119,182	1.52	117,723	0.92
MADRID	216,225	0.24	223,845	0.69	225,574	1.69	237,719	1.61
MURCIA	48,310	0.00	48,085	0.00	50,822	0.96	51,075	0.95
NAVARRA	30,050	0.00	29,495	0.00	29,435	0.30	29,729	0.35
PAIS VASCO	97,910	0.00	94,554	0.08	94,046	0.36	95,731	0.42
LA RIOJA	10,423	0.00	10,200	0.00	9,946	0.31	9,763	0.33
VALENCIA	178,608	0.26	175,766	0.79	172,603	1.37	175,006	2.63
TOTAL	**1,608,101**	0.08	**1,613,141**	**0.24**	**1,657,981**	0.95	**1,681,945**	**1.55**

Source: Estadística Estatal de Centros y Servicios de Transfusión



Graph 2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Graph 3

EVOLUTION OF THE DETECTION OF *TRYPANOSOMA CRUZI* IN DONATIONS

Table 5.

	2004			2005			2006			2007		
AUTONOMOUS COMMUNITIES	**Units tested**	**Confirmed positive units**	**%**	**Units tested**	**Confirmed positive units**	**%**	**Units tested**	**Confirmed positive units**	**%**	**Units tested**	**Confirmed positive units**	**%**
ANDALUCÍA							72	1	139	1,439	19	1.32
ARAGÓN										85	0	0.00
ASTURIAS	304	0					184	0	0.00	201	2	1.00
BALEARES							195		0.00	318	0	0.00
CANARIAS							1,227	16	1.30	4,878	18	0.37
CANTABRIA							81	1	1.23	148	0	0.00
C.MANCHA							20	1	5.00	6	0	0.00
C.LEON							442	0	0.00	380	1	0.26
CATALUÑA				697	6	0.86	4,653	16	0.34	8,194	24	0.29
EXTREMADURA							0	0		5	0	0.00
GALICIA				130			1,812	1	0.06	1,087	0	0.00
MADRID	509	6	1.18	1,539	16	1.04	3,819	37	0.97	3,822	35	0.92
MURCIA							488	3	0.61	483	3	0.62
NAVARRA							87		0.00	104	0	0.00
PAIS VASCO				74	1	1.35	336	5	1.49	403	2	0.50
LA RIOJA							31	0	0.00	32	1	3.13
VALENCIA	465	5	1.08	1,386	13	0.93	2,369	1	0.04	4,599	16	0.35
TOTAL	1,278	11	0.86	3,826	36	0.94	15,816	82	0.52	26,184	121	**0.46**

Source: Estadística Estatal de Centros y Servicios de Transfusión

Anti-*T. cruzi* antibody screening tests used at Transfusion Centers were repeatedly reactive in 0.9% of donations (Appendix Table IV). Prevalence with confirmation tests is **0.46%** (Table 5).

5. PREVENTIVE MEASURES

Prevention of transmission of the disease

1. **General information.** Like with other strategies aimed at preventing the transmission of pathogens through transfusion, it is important that individuals of risk be informed and not donate. Such risk must be notified, and people who are possibly infected should be referred to specific detection units or to their primary care centers.
2. **Detection of carriers,** including rigorous control of women with childbearing potential or who are pregnant, and newborns of mothers from endemic areas is important in order to minimize possible infected donors in the medium-and long-terms.
3. **Pre-donation advising.** Before donating, individuals in any of the risk categories should be carefully evaluated during the medical interview. The first step is the unequivocal identification of the future donor, his/her place of origin and prior places of residence, as well as regular or sporadic future visits to risk areas. It might be advisable to do laboratory tests before accepting them as donors. To the extent possible, it must be guaranteed that this is an altruistic donation by informing that the healthcare system offers simple detection and control methods outside of blood donation settings. In any case, the healthcare staff must know and apply, at a minimum and perfectly, the selection and exclusion.

   The pre-donation interview is a good opportunity to provide relevant information specific to the donor and his/her environment. It is important to remember that very frequently these people may also constitute a risk of other infectious diseases such as malaria or HTLV, for which reason it is often advisable to develop procedures that take into account all the known.

4. **Exclusion of donors of risk.** This is probably the most effective measure, and the only one available to centers with only a few donors with these characteristics. It may also be effective when, even if adequate screening techniques are available, the epidemiological data in the area render this measure appropriate (for example, when there are many donors from an area with an especially high prevalence). In any case, these measures should be accompanied by an evident and clear rationale that will avoid interpretations leading to discrimination or marginalization of the communities involved. (Basic criteria for the selection of donors of blood and blood component. Ministerio de Sanidad y Consumo, 2006)[43]

5. **Management of positive donors, negative donors, and inconclusive situations.** All individuals who theoretically are carriers must be notified and guided to treatment; they should also be educated, with their surroundings, about the epidemiological characteristics of the infection, with the purpose of avoiding spread (transfusional or vertical).

6. **Type of donation and manufacture of components. Donation processing: Leukocyte reduction**.

   Parasite viability studies in the various blood components, as well as diverse but few retrospective studies conducted with recipients of products from carrier donors, show that the risk of transmission varies significantly (and is more frequent through packed platelets). Therefore, performing pre-storage leukocyte reduction, not producing units of platelets obtained from whole blood donated by certain donors, and not including them in platelet apheresis programs may be considered complementary—but not exclusive—safety measures. The latter measures may be considered complementary strategies until the actual efficacy of the other measures is learned.

7. **Look-back studies.** It is important to study all the recipients involved so that they can receive early treatment, especially girls, youth, or women with childbearing potential.

8. Laboratory screening and confirmation tests

New detection methods with even better sensitivity and specificity levels are expected in the future. In this case, it would be justified to re-analyze risk donors with previously negative results. It would be desirable to incorporate such tests in existing external control and assessment programs if they provide continuous monitoring of their efficacy; the purpose is to determine which are most appropriate for use in Transfusional Medicine or which, for instance, provide information about the potential residual risk of transmission after implementation.

One issue not yet resolved is whether to perform selective or universal screening. The disadvantages of selective screening derive from the difficulty or impossibility of detecting all individuals at risk through a pre-interview, the logistic difficulty of managing donors with frequent changes of place of residence or visits to risk areas, the detection of infected local donors not included in the exclusion criteria, or even donors born in Europe from infected mothers.

The disadvantages of universal screening are, obviously, the high cost of testing and the secondary costs caused by a greater permissibility at the interview, with the risk of accepting donors with risk of other emerging infections. Universal screening has several logistic advantages, such a routinely analyzing donors with risk every time new generations of ELISA with higher sensitivity are instated. In any case, it may be justified to consider universal screening based on the total number of existing carriers in Spain (>50,000), or in Autonomous Communities with a large number of emigrants from endemic areas. In general, universal screening eliminates many logistical problems and minimizes the possibility of error in routine sample management, for which reason it could become an adequate option for a country like Spain, where everything suggests that this is a firmly implanted emerging infection.

To illustrate, Appendix shows some maps (AABB, 2008)[44] of the findings of the Chagas Biovigilance Network of the American Association of Blood Banks (in this country, approximately 65% of donations are screened with the Ortho ELISA method approved in December of 2006, and confirmed with RIPA).

Donor Selection and Exclusion Criteria

The following will not be accepted as blood donors No:

1. Individuals who have had Chagas disease or infection with the parasite will be definitively excluded as blood donors.
2. Individuals who were born, resided, received transfusions, or whose mothers were born, resided or received transfusions in *T. cruzi* endemic areas: Mexico, Central America and South America (Basic criteria for the selection of donors of blood and blood component. Ministerio de Sanidad y Consumo, 2006)[43].
3. Visitors to endemic areas will be individually assessed taking into consideration the epidemiological characteristics of the area visited.

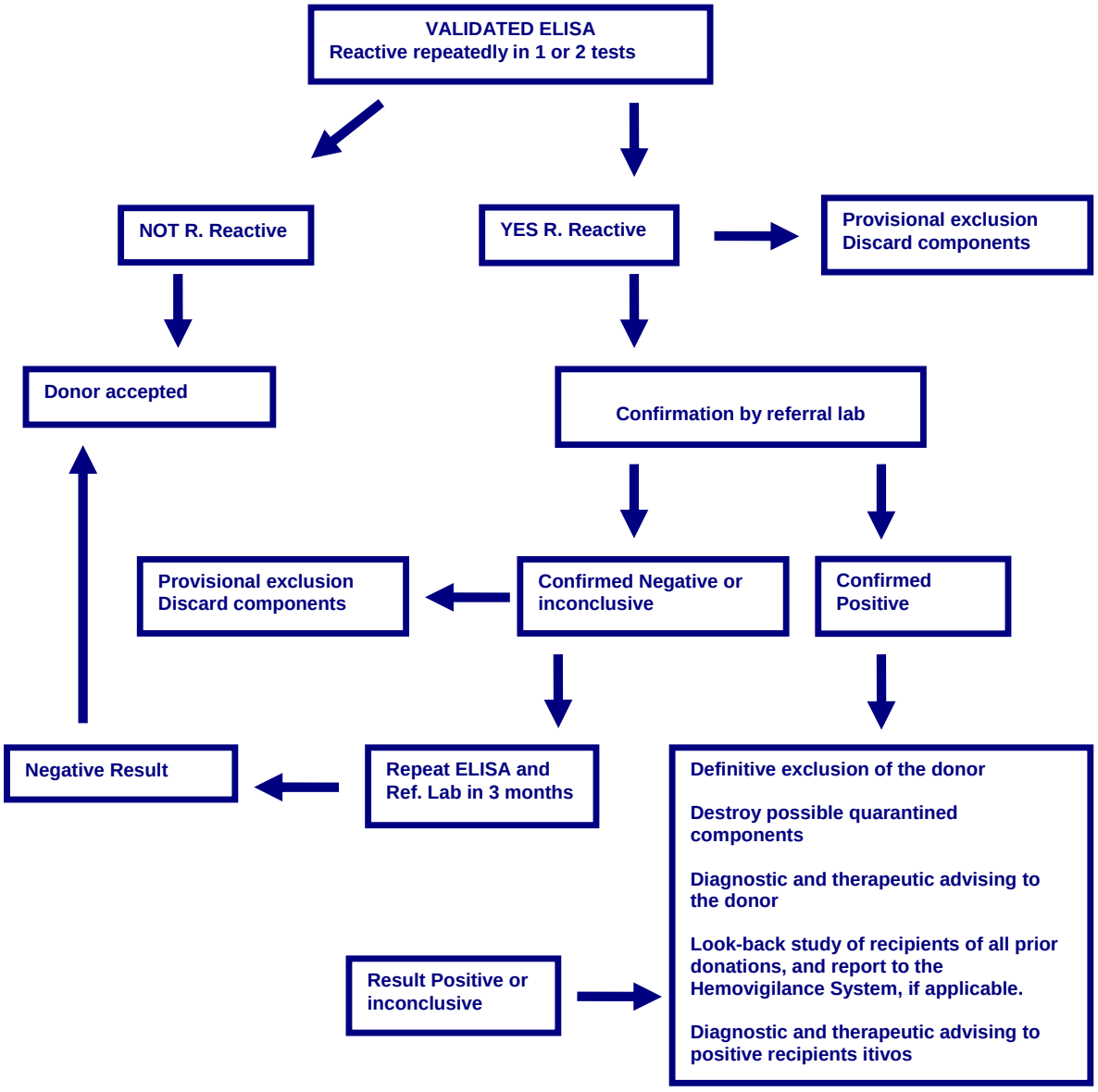
In case 2 and in selected instances of case 3, individuals may be accepted if a validated test for the detection of antibodies against *Trypanosoma cruzi* done at least six months after the last possible exposure to the parasite is negative.

In all other cases, the centers will have available updated procedures for the computerized management of donors and donations to ensure the recording of individuals of risk in order to avoid future donations from excluded donors and the automatic disqualification of components that are still available or quarantined.

Each Transfusion Center must have updated epidemiological data about the specific situation of its area of influence and implement additional safety measures if assessment calls for it.

It must be kept in mind that often the endemic areas for this parasite are also endemic for other infectious agents (malaria, HTLV, etc.); for this reason, when developing exclusion criteria and laboratory screening, there should be a global coordinated strategy so as not to ignore these risks.

Chagas Disease Screening Algorithm. Proposal



6. ESTIMATION OF RISK IN SPAIN

As an attempt to estimate the risk of transmission of the infection via transfusion in Spain, based on the data provided, the following calculations are done:

1. <u>Chagas disease carrier population</u>

Applying the calculations proposed by Schmunis (*Schmunis, 2007)*[45] based on the prevalence per thousand donors in the respective countries of origin, the following is collected:

- Number of residents in Spain who emigrated from endemic countries (INE data).
- Prevalence of Chagas disease in each of the countries of origin (Schmunis and Castro Izaguirre data).
- Results.

Estimated infected individuals according to provenance. National total

	ORIGIN	Estimated infected
1	BOLIVIA	35,509
2	ARGENTINA	7,120
3	COLOMBIA	3,367
4	PARAGUAY	3,003
5	BRAZIL	924
6	ECUADOR	843
7	VENEZUELA	748
8	CHILE	545
9	URUGUAY	301
10	HONDURAS	262
11	PERU	248
12	MEXICO	115
13	EL SALVADOR	75
14	GUATEMALA	44
15	NICARAGUA	16
16	COSTA RICA	12
17	PANAMA	2
TOTAL		**53,134**

In order to learn the contribution of each country of provenance to the total computation, the weight of each on the national total is calculated.

	ORIGIN	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	35,509	66.829
2	ARGENTINA	42,629	80.229
3	COLOMBIA	45,996	86.566
4	PARAGUAY	48,999	92.217
5	BRAZIL	49,923	93.956
6	ECUADOR	50,766	95.543
7	VENEZUELA	51,514	96.951
8	CHILE	52,059	97.976
9	URUGUAY	52,360	98.543
10	HONDURAS	52,622	99.036
11	PERU	52,870	99.503
12	MEXICO	52,985	99.719
13	EL SALVADOR	53,060	99.860
14	GUATEMALA	53,104	99.943
15	NICARAGUA	53,120	99.973
16	COSTA RICA	53,132	99.996
17	PANAMA	53,134	100
TOTAL		**53,134**	

As shown on the table, the first ten countries contribute 99% of potentially infected carriers.

Due to the heterogeneity in the emigrant population distribution in the various Autonomous Communities, the same data are studied for each Autonomous Community (Appendix Table V).

2. Estimation of the number of potentially infected donations

As an attempt to achieve a higher precision in the estimation of the risk of transmission via transfusion, the number of potentially infected donations is calculated with the following **formula**:

> **Number of potentially infected donations = [number of donors of risk x prevalence of the disease] x mean number of donations per donor.** (from *Institut de Veille Sanitaire, 2007*)[46]
>
> **Donors of risk:** number of carriers estimated in previous study.
>
> **Prevalence of the disease:** Prevalence per thousand inhabitants and per country of provenance.
>
> **Number of donations per donor:** Datum from statistics of the National Information System of the National System for Transfusion Safety.

2.1 If we assume that 100% of the immigrant population donates blood, the estimation of the number of donations **potentially of risk** according to the formula would be **70,509.**

The following table shows this in decreasing order of each Autonomous Community and the rates per 1000 donations.

Table 6

AUTONOMOUS COMMUNITY	NUMBER OF POTENTIALLY INFECTED DONATIONS	POTENTIALLY INFECTED DONATIONS X THOUSAND
CATALUÑA	14,752	53
MADRID	13,977	59
C.VALENCIANA	9,929	57
ANDALUCIA	7,526	28
MURCIA	5,244	103
BALEARES	3,772	98
PAIS VASCO	3,610	38
C.MANCHA	2,734	32
CANARIAS	2,130	34
C.LEON	1,605	17
NAVARRA	1,355	46
GALICIA	1,152	10
LA RIOJA	726	74
ARAGON	635	15
ASTURIAS	487	12
EXTREMADURA	439	10
CANTABRIA	436	18
TOTAL	**70,509**	42

2.2 If the study is based on *T. cruzi* tests done on blood donations in 2007, the results are:

Table 7

AUTONOMOUS COMMUNITY	NUMBER OF POTENTIALLY INFECTED DONATIONS	POTENTIALLY INFECTED DONATIONS X THOUSAND (2)
CATALUÑA	339	**1.21**
C.VALENCIANA	240	**1.37**
CANARIAS	146	**2.35**
MADRID	128	**0.54**
ANDALUCIA	83	**0.31**
GALICIA	30	**0.26**
MURCIA	30	**0.58**
PAÍS VASCO	28	**0.3**
BALEARES	19	**0.5**
C-LEÓN	15	**0.15**
ASTURIAS	6	**0.15**
NAVARRA	5	**0.18**
CANTABRIA	5	**0.19**
EXTREMADURA	2	**0.05**
LA RIOJA	2	**0.21**
ARAGÓN	1	**0.02**
C-MANCHA	*	*****
TOTAL	**1079**	**0.67**

The wide heterogeneity found between the various Autonomous Communities may justify adopting different measures according to risk.

SUMMARY

1. CHAGAS DISEASE IN NON-ENDEMIC COUNTRIES

Changing migration patterns have caused substantial changes in the epidemiology of American trypanosomiasis or Chagas disease, which has up to the present been associated with rural, impoverished areas in Latin American countries; now, it has become a disease diagnosed in the large urban centers of the Americas, as well as in countries in other continents. In Spain, demographic data suggest a sustained growth of the resident foreign population from 2000 to 2008. Latin American immigration in late 2007 reached 1,600,000 citizens. It is known that a percentage of these immigrants have Chagas disease in some of its chronic forms. **Imported Chagas disease thus constitutes a new public health problem in non-endemic countries.**

2. CHAGAS DISEASE AND TRANSFUSION

Transfusion is the **second most common cause** of transmission of the disease, after vectorial transmission. Infectious capacity through blood and blood components depends on several factors: type and quantity of the component transfused, the strain of the parasite, the presence of parasitemia at the time of donation, the immune status of the recipient, and whether or not screening tests are done. It is possible that some cases of post-transfusional Chagas disease may not be diagnosed. Since 2005, five cases of Chagas disease secondary to transfusion have been reported in Spain; all were caused by transfusion of packed platelets in immunosuppressed patients.

3. DETECTION/CONFIRMATION TESTING FOR *TRYPANOSOMA CRUZI* INFECTION

Diagnosis of the possible infection is based on the determination of specific anti-*Trypanosoma cruzi* antibodies. Despite technological advances, no serologic assay has a 100% sensitivity and specificity; thus, confirmation is possible only if at least two techniques with different principles and antigens agree. **There is no consensus for establishing a common reference technique.**

4. INCIDENCE OF *TRYPANOSOMA CRUZI* IN BLOOD DONATIONS IN SPAIN

There was a significant increase in the number of screening tests done: from 0.08% in 2004 in only a few Autonomous Communities, to 1.5% (18-fold increase) in 2007, in most Autonomous Communities. The results from these tests offer the more significant datum that 0.9% is repeatedly positive, resulting in a **mean seroprevalence of 0.46% in 2007,** with an important **variability between the various Autonomous Communities**.

5. PREVENTIVE MEASURES

Potential measures are described, including general ones such as: *notifying the population of risk, detecting carriers, providing advice before the blood donation, excluding donors of risk, performing leukocyte reduction in blood components, etc*. Consideration is given to the advantages and disadvantages of screening tests only in the populations deemed of risk (as defined in the current recommendations), or universal screening. Exclusion criteria effective in the country and an algorithm for action after screening tests are presented.

6. ESTIMATION OF RISK IN SPAIN

Based on our knowledge of the migrant population and the estimates of potentially infected donations (done, for comparison purposes, with calculations similar to those used by the French blood agency), the results suggest that in our country there are approximately **53,000 potential carriers**, with a rate of potentially infected donations ranging between 0.02 and 2.35 (per thousand), which reflects a great heterogeneity between Autonomous Communities.

7. CONCLUSION

No countrywide measure beyond those already established is advisable; however, an assessment of universal screening is recommended in the Autonomous Communities with a higher risk and according to the characteristics of the Transfusion Center.

APPENDICES

Table I. Technical characteristics of the main serologic diagnostic tests for *Trypanosoma cruzi* infection performed in Spain

TEST	ANTIGEN	CONJUGATE	TIME	CUT OFF	POSITIVE	NEGATIVE	UNCERTAIN
IIF-CNM	Epimastigotes (T, Mc, Dm28)	Anti-IgG Hum (FITC)	90 min	1/40	≥1/40	<1/40	1/20, ± 1/40
ELISA-CNM	Soluble extract (T, Mc, Dm28)	Anti-IgG Hum (Biot, Strep-HRP)	4 h	mCN+4SD	OD≥CO	OD<[0.8xCO]	OD>0.8CO to <CO
ORTHO® *T.cruzi* ELISA Test System	Total extract	Anti-IgG Hum (HRP)	3 h	mCPx0.460	OD/CO≥1	OD/CO<1	Option does not exist
CERTEST Chagas ELISA Test	Total extract (Tulahuen, Mn)	Anti-IgG Hum (HRP)	2 h	[mCP+mCN]x0.35	OD>[1.1xCO]	OD<[0.9xCO]	OD=CO±10%CO
BLK *T. cruzi* IgG ELISA	Total extract	Anti-IgG Hum (HRP)	40 min	0.200	OD>0.220	OD<0.180	OD≥0.180 to <0.220
Elisa cruzi BioMérieux	Total extract (Y)	Anti-IgG Hum (HRP)	2 h	mCN+0.250	OD/CO≥1	OD/CO<0.8	OD/CO≥0.8 to <1
Bioelisa Chagas Biokit	TcD, TcE, Pep2, TcLo1.2	Anti-IgG Hum, anti-IgM Hum (HRP)	90 min	mCN+0.300	OD/CO≥1	OD/CO<0.9	OD/CO≥0.9 to <1
NovaLisa™ Chagas (*T. cruzi*) IgG ELISA	TcD, TcE, Pep2, TcLo1.2	Protein A	2 h	mC CO	[ODx10]/CO>11	[ODx10]/CO<9	[ODx10]/CO≥9 to ≤11
OnSite Chagas Ab Combo Rapid Test	Recombinant antigen	Protein A	30 min	Not established	Two bands	Control band	Option does not exist
Stick Chagas (ICT Operon)	TcD, TcE, Pep2, SAPA		30 min	Not established	Two bands	Control band	Option does not exist
Chagas Check-1 Gernon	Recombinant antigen		15 min	Not established	Two bands	Control band	Option does not exist

CO = Cut off
OD = Optical density
NC = Negative control
PC = Positive control
SD = Standard deviation

Table II. Commercial tests available in Spain

COMPANY	NAME OF TEST	TYPE OF ANTIGEN	SENSITIVITY	SPECIFICITY	REF.
Inverness Medical	Inmunofluor Chagas (Biocientífica)	Epimastigotes	NS	NS	
LabClinics	Biognost® IFA	Epimastigotes	NS	NS	
Innogenetics Ibérica	IFA Kit Tripanosomiasis (MarDx)	Epimastigotes Corpus Christi	NS	NS	
Vitros	*T. cruzi* IgG ELISA (Cellabs)	Total antigen	NS	NS	
Johnson&Johnson	ORTHO® *T.cruzi* ELISA Test System	Total antigen	100	100	a
Abbot Diagnostic	CERTEST Chagas ELISA Test	Total antigen	100	100	a
BLK Diagnostics	BLK *T.cruzi* IgG ELISA	Alkaline total extract (Y strain)	NS	NS	
BioMérieux España	Elisa cruzi (Chagas disease)	Total antigen	100	100	a
Izasa	Bioelisa Chagas Biokit	Recombinant antigen	100	97.4-99.5	a
Diasorin / Radim Ibérica / Siemens Healthcare Diagnostics	NovaLisa™ Chagas (*T. cruzi*) IgG ELISA	Recombinant antigen	86.7	91	a
Inverness Medical / Laboratorios Leti / CTK Biotech	OnSite Chagas Ab Combo Rapid Test	Recombinant antigen	92.9	100	a
Operon SA	*Stick Chagas*	Recombinant antigen	99	95	a
RAL Técnica para el Laboratorio, S.A.	Chagas Check-1 Gernon	Recombinant antigen	98.1	98.4	a

NS= Not specified
[a] Manufacturer's data

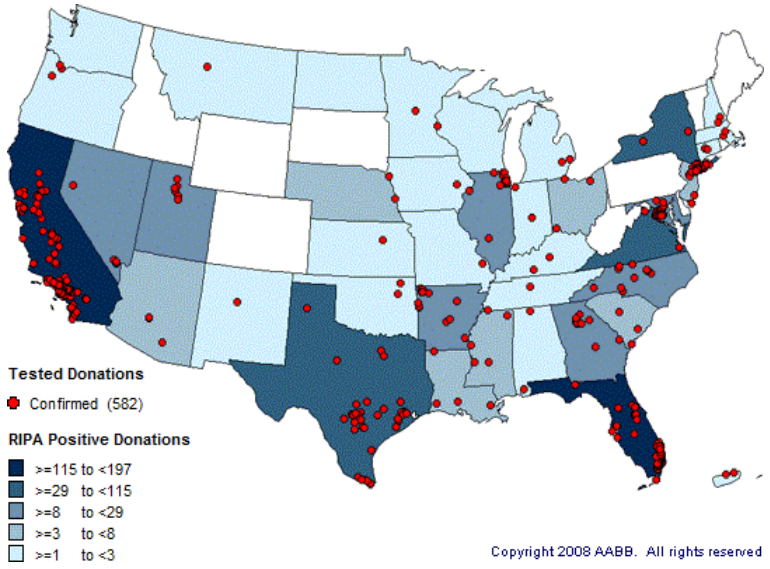
Table III. Distribution of foreign population per Autonomous Community

	TOTAL POPULATION	TOTAL FOREIGNERS		EUROPEANS		AFRICANS		AMERICAS		ASIANS		OCEANIA		STATELESS
TOTAL	45,200,737	4,519,554	10%	1,895,727	4%	806,795	2%	1,594,338	4%	219,843	0.5%	2,271	0.01%	580
ANDALUCIA	8,050,461	531,827	7%	278,276	3%	110,985	1%	126,431	2%	15,842	0.2%	261	0.00%	32
ARAGÓN	1,296,655	124,404	10%	60,024	5%	27,682	2%	32,621	3%	4,010	0.3%	39	0.00%	28
ASTURIAS (PRINCIPADO DE)	1,074,862	32,720	3%	11,481	1%	2,832	0%	17,236	2%	1,140	0.1%	29	0.00%	2
BALEARS (ILLES)	1,030,650	190,170	18%	100,934	10%	25,495	2%	58,132	6%	5,453	0.5%	155	0.02%	1
CANARIAS	2,025,951	250,736	12%	135,790	7%	25,052	1%	77,502	4%	12,231	0.6%	75	0.00%	86
CANTABRIA	572,824	26,795	5%	9,929	2%	2,192	0%	13,790	2%	857	0.1%	27	0.00%	0
CASTILLA Y LEÓN	2,528,417	119,781	5%	57,249	2%	16,886	1%	41,686	2%	3,904	0.2%	29	0.00%	27
CASTILLA-LA MANCHA	1,977,304	159,637	8%	81,423	4%	27,540	1%	47,295	2%	3,314	0.2%	31	0.00%	34
CATALUÑA	7,210,508	972,507	13%	274,252	4%	253,016	4%	357,707	5%	87,028	1.2%	456	0.01%	48
COMUNITAT VALENCIANA	4,885,029	732,102	15%	435,155	9%	89,245	2%	183,094	4%	23,999	0.5%	563	0.01%	46
EXTREMADURA	1,089,990	29,210	3%	10,718	1%	10,155	1%	7,534	1%	794	0.1%	4	0.00%	5
GALICIA	2,772,533	81,442	3%	29,846	1%	7,316	0%	42,117	2%	2,060	0.1%	78	0.00%	25
MADRID (COMUNIDAD DE)	6,081,689	866,910	14%	296,390	5%	101,108	2%	421,844	7%	47,041	0.8%	373	0.01%	154
MURCIA (REGION DE)	1,392,117	201,700	14%	53,345	4%	63,878	5%	81,163	6%	3,222	0.2%	19	0.00%	73
NAVARRA (C. FORAL DE)	605,876	55,921	9%	17,073	3%	11,070	2%	26,840	4%	909	0.2%	27	0.00%	2
PAIS VASCO	2,141,860	98,524	5%	28,341	1%	16,822	1%	48,324	2%	4,929	0.2%	93	0.00%	15
RIOJA (LA)	308,968	36,825	12%	14,543	5%	8,413	3%	10,854	4%	3,004	1.0%	11	0.00%	0
Ceuta	76,603	3,016	4%	214	0%	2,618	3%	103	0%	81	0.1%	0	0.00%	0
Melilla	69,440	5,327	8%	744	1%	4,490	6%	65	0%	25	0.0%	1	0.00%	2

Source: Instituto Nacional de Estadística

Table IV. Screening for anti *T cruzi* in Spain. Prevalence and type of test used.
Cumulative from 2002 to May 2007

AUTONOMOUS COMMUNITIES	NUMBER OF DONORS TESTED	NUMBER OF DONORS ANTIBODY-POSITIVE	PREVALENCE ANTI-*T CRUZI*	NUMBER OF DONATIONS IN THE PERIOD	% DONATIONS TESTED / TOTAL DONATIONS	TYPE OF TEST	TEST DONE PRE/POST-DONATION	DATE BEGINNING TEST
Andalucía	50	0	0.00%			Certest -Abbott	pre	Mar-07
Aragón	0	0		2,292	0.00%	Immunochromatography (Operon)	post	May-07
Asturias	933	1	0,11%	180,810	0.52%	DiaMed-ID PaGIA	pre	Jan-03
Baleares	195	0	0,00%	38,475	0.49%	DiaMed-ID PaGIA	post	Apr-06
Canarias	5,737	32	0.56%	80,697	7.11%	ELISA Dade Behring	post	Mar-06
Cantabria *	190	3	1.58%	20,265	0.94%	DiaMed-ID PaGIA	post	Jun-06
Castilla y León	490	1	0.20%	83,028	0.59%	DiaMed-ID PaGIA	pre	Jun-04
Castilla La Mancha	NA	NA		NA		Donor deferral. No testing	NA	NA
Cataluña	5,951	32	0.54%	464,712	1.28%	DiaMed-ID PaGIA / ELISA Biokit	post	Sep-05
Comunidad Valenciana	5,337	51	0.96%	520,215	1.03%	DiaMed-ID PaGIA +IFI (Biocientífica)	post	Sep-04
Extremadura	NA	NA		NA		Donor deferral. No testing	NA	NA
Galicia	2,470	16	0.5%	182,295	1.35%	DiaMed-ID PaGIA	post	Nov-05
Madrid (CTCM-CRE)	6,990	108	1.55%	639,373	1.09%	ELISA in house /DiaMed-ID PaGIA	pre / post	02/2002 08/2005
Murcia	707	4	0.57	66,963	1.05	ELISA Ortho	post	Feb-2006
Navarra	117	0	0.00%	39,476	0.30%	DiaMed-ID PaGIA	pre	Feb-06
País Vasco	683	7	1.02%	154,155	0.44%	DiaMed-ID PaGIA / ELISA Biokit/ ELISA Certest -Abbott	pre	Oct-05
Rioja	46	0	0.00%	12,913	0.36%	DiaMed-ID PaGIA /	post	Jan-06
Total	29.846	255	0.9%	2.484.377	1.2%			


Tested Donations
Confirmed (582)
RIPA Positive Donations
>=115 to <197
>=29 to <115
>=8 to <29
>=3 to <8
>=1 to <3
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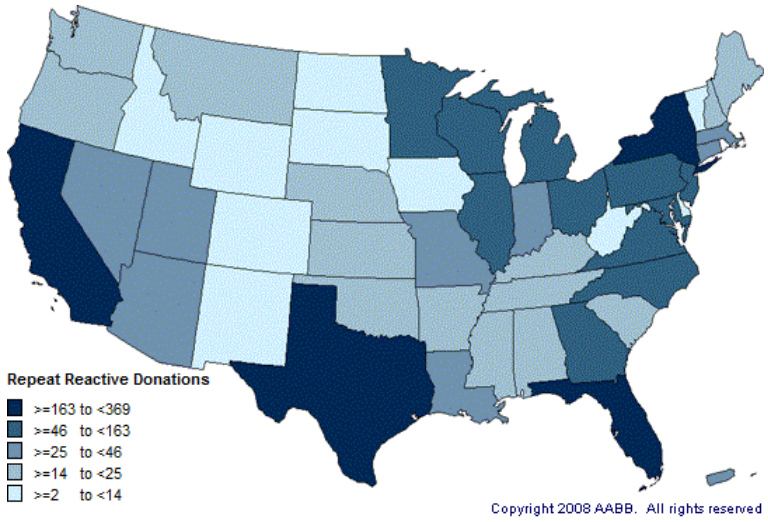
Repeat Reactive Donations
>=163 to <369
>=46 to <163
>=25 to <46
>=14 to <25
>=2 to <14
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ESTIMATED INFECTED POPULATION ACCORDING TO PROVENANCE AUTONOMOUS COMMUNITIES

Table V

ANDALUCIA

MEAN DISTRIBUTION

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1.	BOLIVIA	3,268	3,268	59.930
2	ARGENTINA	1,252	4520	82.890
3	PARAGUAY	400	4920	90.226
4	COLOMBIA	250	5170	94.810
5	BRAZIL	89	5259	96.442
6	VENEZUELA	57	5316	97.488
7	ECUADOR	46	5362	98.331
8	CHILE	38	5400	99.028
9	URUGUAY	19	5419	99.376
10	PERU	9	5428	99.542
11	MEXICO	9	5437	99.707
12	HONDURAS	6	5443	99.817
13	GUATEMALA	4	5447	99.890
14	EL SALVADOR	3	5450	99.945
15	NICARAGUA	1	5451	99.963
16	COSTA RICA	1	5452	99.982
17	PANAMA	0	5452	99.982
	TOTAL	**5,453**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
99.028	99.38	99.48

ARAGÓN

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1.	BOLIVIA	179	179	37.214
2	ARGENTINA	108	287	59.667
3	COLOMBIA	86	373	77.547
4	ECUADOR	23	396	82.328
5	BRAZIL	19	415	86.279
6	PARAGUAY	15	430	89.397
7	VENEZUELA	13	443	92.100
8	CHILE	11	454	94.387
9	HONDURAS	7	461	95.842
10	NICARAGUA	5	466	96.881
11	PERU	4	470	97.713
12	EL SALVADOR	4	474	98.545
13	URUGUAY	4	478	99.376
14	MEXICO	2	480	99.792
15	GUATEMALA	1	481	100
16	COSTA RICA	0	481	100
17	PANAMA	0	481	100
	TOTAL	**481**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
94.38	95.84	96.67

ASTURIAS

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1.	BOLIVIA	78	78	24.606
2	ARGENTINA	73	151	47.634
3	PARAGUAY	70	221	69.716
4	COLOMBIA	35	256	80.757
5	BRAZIL	23	279	88.013
6	VENEZUELA	12	291	91.798
7	ECUADOR	8	299	94.322
8	PERU	8	307	96.845
9	CHILE	5	312	98.423
10	URUGUAY	3	315	99.369
11	MEXICO	2	317	100
12	HONDURAS	0	317	100
13	EL SALVADOR	0	317	100
14	NICARAGUA	0	317	100
15	GUATEMALA	0	317	100
16	COSTA RICA	0	317	100
17	PANAMA	0	317	100
	TOTAL	**318**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
95.60	96.54	96.54

BALEARES

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1.	BOLIVIA	1226	1226	55.931
2	ARGENTINA	602	1828	83.394
3	COLOMBIA	119	1947	88.823
4	PARAGUAY	95	2042	93.157
5	CHILE	36	2078	94.799
6	BRAZIL	32	2110	96.259
7	URUGUAY	30	2140	97.628
8	ECUADOR	27	2167	98.859
9	VENEZUELA	15	2182	99.544
10	PERU	4	2186	99.726
11	HONDURAS	2	2188	99.818
12	MEXICO	2	2190	99.909
13	EL SALVADOR	1	2191	99.954
14	GUATEMALA	1	2192	100
15	NICARAGUA	0	2192	100
16	COSTA RICA	0	2192	100
17	PANAMA	0	2192	100
	TOTAL	**2,193**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
98.85	99.54	99.58

CANARIAS

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1.	BOLIVIA	663	663	37.649
2	ARGENTINA	493	1156	65.645
3	COLOMBIA	258	1414	80.295
4	VENEZUELA	154	1568	89.040
5	PARAGUAY	65	1633	92.731
6	URUGUAY	37	1670	94.832
7	CHILE	36	1706	96.877
8	BRAZIL	27	1733	98.410
9	ECUADOR	12	1745	99.091
10	HONDURAS	4	1749	99.319
11	PERU	4	1753	99.546
12	MEXICO	3	1756	99.716
13	GUATEMALA	3	1759	99.886
14	EL SALVADOR	1	1760	99.943
15	COSTA RICA	1	1761	100
16	NICARAGUA	0	1761	100
17	PANAMA	0	1761	100
	TOTAL	**1,760**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
98.41	99.09	99.32

CANTABRIA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	91	91	34.733
2	COLOMBIA	47	138	52.672
3	ARGENTINA	46	184	70.229
4	PARAGUAY	40	224	85.496
5	BRAZIL	13	237	90.458
6	VENEZUELA	8	245	93.511
7	PERU	5	250	95.420
8	ECUADOR	4	254	96.947
9	CHILE	3	257	98.092
10	MEXICO	2	259	98.855
11	URUGUAY	1	260	99.237
12	HONDURAS	1	261	99.618
13	GUATEMALA	1	262	100
14	EL SALVADOR	0	262	100
15	NICARAGUA	0	262	100
16	COSTA RICA	0	262	100
17	PANAMA	0	262	100
	TOTAL	**261**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
96.55	96.93	97.32

C-LA MANCHA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	1,383	1383	72.905
2	PARAGUAY	148	1531	80.706
3	COLOMBIA	142	1673	88.192
4	ARGENTINA	138	1811	95.467
5	ECUADOR	27	1838	96.890
6	BRAZIL	15	1853	97.681
7	VENEZUELA	14	1867	98.419
8	CHILE	9	1876	98.893
9	PERU	7	1883	99.262
10	HONDURAS	5	1888	99.526
11	URUGUAY	4	1892	99.736
12	MEXICO	2	1894	99.842
13	EL SALVADOR	2	1896	99.947
14	NICARAGUA	1	1897	100
15	GUATEMALA	0	1897	100
16	COSTA RICA	0	1897	100
17	PANAMA	0	1897	100
	TOTAL	**1,899**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
98.89	99.05	99.26

C- LEÓN

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	632	632	59.398
2	COLOMBIA	130	762	71.617
3	ARGENTINA	123	885	83.177
4	PARAGUAY	55	940	88.346
5	BRAZIL	50	990	93.045
6	ECUADOR	18	1008	94.737
7	VENEZUELA	18	1026	96.429
8	HONDURAS	13	1039	97.650
9	CHILE	8	1047	98.402
10	PERU	6	1053	98.966
11	URUGUAY	4	1057	99.342
12	MEXICO	4	1061	99.718
13	GUATEMALA	2	1063	99.906
14	EL SALVADOR	1	1064	100
15	NICARAGUA	0	1064	100
16	COSTA RICA	0	1064	100
17	PANAMA	0	1064	100
	TOTAL	**1,063**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
97.65	98.40	98.87

CATALUÑA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	8,959	8959	69.839
2	ARGENTINA	1,716	10,675	83.216
3	COLOMBIA	552	11,227	87.519
4	PARAGUAY	541	11,768	91.737
5	BRAZIL	205	11,973	93.335
6	CHILE	196	12,169	94.863
7	ECUADOR	161	12,330	96.118
8	HONDURAS	138	12,468	97.194
9	VENEZUELA	121	12,589	98.137
10	URUGUAY	96	12,685	98.885
11	PERU	65	12,750	99.392
12	MEXICO	36	12,786	99.673
13	EL SALVADOR	26	12,812	99.875
14	GUATEMALA	9	12,821	99.945
15	COSTA RICA	4	12,825	99.977
16	NICARAGUA	2	12,827	99.992
17	PANAMA	1	12,828	100
	TOTAL	**12,828**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
97.194	98.137	98.88

EXTREMADURA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	148	148	61.925
2	ARGENTINA	35	183	76.569
3	COLOMBIA	19	202	84.519
4	BRAZIL	16	218	91.213
5	PARAGUAY	8	226	94.561
6	HONDURAS	3	229	95.816
7	ECUADOR	2	231	96.653
8	VENEZUELA	2	233	97.490
9	CHILE	2	235	98.326
10	URUGUAY	1	236	98.745
11	PERU	1	237	99.163
12	MEXICO	1	238	99.582
13	EL SALVADOR	1	239	100
14	GUATEMALA	0	239	100
15	NICARAGUA	0	239	100
16	COSTA RICA	0	239	100
17	PANAMA	0	239	100
	TOTAL	**236**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
97.50	98.33	98.74

GALICIA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	ARGENTINA	251	251	30.461
2	BOLIVIA	221	472	57.282
3	COLOMBIA	99	571	69.296
4	BRAZIL	84	655	79.490
5	PARAGUAY	64	719	87.257
6	VENEZUELA	52	771	93.568
7	URUGUAY	26	797	96.723
8	CHILE	11	808	98.058
9	PERU	5	813	98.665
10	ECUADOR	3	816	99.029
11	MEXICO	3	819	99.393
12	HONDURAS	2	821	99.636
13	GUATEMALA	2	823	99.879
14	EL SALVADOR	1	824	100
15	NICARAGUA	0	824	100
16	COSTA RICA	0	824	100
17	PANAMA	0	824	100
	TOTAL	**823**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
98.05	98.54	98.78

MADRID

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	8,464	8464	69.634
2	PARAGUAY	990	9454	77.779
3	ARGENTINA	921	10375	85.356
4	COLOMBIA	794	11169	91.888
5	ECUADOR	271	11440	94.118
6	BRAZIL	185	11625	95.640
7	VENEZUELA	169	11794	97.030
8	CHILE	110	11904	97.935
9	PERU	108	12012	98.824
10	HONDURAS	45	12057	99.194
11	MEXICO	32	12089	99.457
12	EL SALVADOR	25	12114	99.663
13	URUGUAY	19	12133	99.819
14	GUATEMALA	14	12147	99.934
15	COSTA RICA	4	12151	99.967
16	NICARAGUA	3	12154	99.992
17	PANAMA	1	12155	100
	TOTAL	**12,154**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
97.93	98.31	98.47

MURCIA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	3,045	3045	87.100
2	ARGENTINA	119	3164	90.503
3	PARAGUAY	107	3271	93.564
4	ECUADOR	94	3365	96.253
5	COLOMBIA	81	3446	98.570
6	BRAZIL	18	3464	99.085
7	VENEZUELA	9	3473	99.342
8	CHILE	8	3481	99.571
9	HONDURAS	5	3486	99.714
10	URUGUAY	3	3489	99.800
11	PERU	2	3491	99.857
12	MEXICO	2	3493	99.914
13	GUATEMALA	2	3495	99.971
14	EL SALVADOR	1	3496	100.000
15	NICARAGUA	1	3497	100.029
16	COSTA RICA	1	3498	100.057
17	PANAMA	0	3498	100.057
	TOTAL	**3,496**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
96.57	99.71	99.80

NAVARRA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	515	515	75.959
2	COLOMBIA	53	568	83.776
3	ARGENTINA	45	613	90.413
4	ECUADOR	23	636	93.805
5	BRAZIL	14	650	95.870
6	PARAGUAY	6	656	96.755
7	CHILE	6	662	97.640
8	VENEZUELA	5	667	98.378
9	PERU	4	671	98.968
10	MEXICO	2	673	99.263
11	EL SALVADOR	2	675	99.558
12	URUGUAY	1	676	99.705
13	HONDURAS	1	677	99.853
14	GUATEMALA	1	678	100
15	NICARAGUA	0	678	100
16	COSTA RICA	0	678	100
17	PANAMA	0	678	100
	TOTAL	**677**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
98.37	98.67	98.81

PAÍS VASCO

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	1,755	1755	76.338
2	ARGENTINA	146	1901	82.688
3	COLOMBIA	145	2046	88.995
4	PARAGUAY	134	2180	94.824
5	BRAZIL	41	2221	96.607
6	VENEZUELA	23	2244	97.608
7	ECUADOR	15	2259	98.260
8	CHILE	13	2272	98.826
9	HONDURAS	10	2282	99.261
10	PERU	5	2287	99.478
11	MEXICO	4	2291	99.652
12	URUGUAY	3	2294	99.783
13	EL SALVADOR	2	2296	99.870
14	NICARAGUA	2	2298	99.957
15	GUATEMALA	1	2299	100
16	COSTA RICA	0	2299	100
17	PANAMA	0	2299	100
	TOTAL	**2,300**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
98.82	99.26	99.35

LA RIOJA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	387	387	80.625
2	COLOMBIA	39	426	88.750
3	ARGENTINA	33	459	95.625
4	ECUADOR	6	465	96.875
5	BRAZIL	5	470	97.917
6	PARAGUAY	4	474	98.750
7	VENEZUELA	3	477	99.375
8	CHILE	1	478	99.583
9	URUGUAY	1	479	99.792
10	PERU	1	480	100
11	HONDURAS	0	480	100
12	MEXICO	0	480	100
13	EL SALVADOR	0	480	100
14	GUATEMALA	0	480	100
15	NICARAGUA	0	480	100
16	COSTA RICA	0	480	100
17	PANAMA	0	480	100
	TOTAL	**481**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
99.58	99.79	99.58

VALENCIA

	ORIGIN	ESTIMATED INFECTED	CUMULATIVE TOTAL	% OF TOTAL
1	BOLIVIA	4,496	4496	67.024
2	ARGENTINA	1,019	5,515	82.215
3	COLOMBIA	518	6,033	89.937
4	PARAGUAY	261	6,294	93.828
5	ECUADOR	103	6,397	95.364
6	BRAZIL	88	6,485	96.676
7	VENEZUELA	73	6,558	97.764
8	CHILE	52	6,610	98.539
9	URUGUAY	49	6,659	99.270
10	HONDURAS	20	6,679	99.568
11	PERU	10	6,689	99.717
12	MEXICO	9	6,698	99.851
13	EL SALVADOR	5	6,703	99.925
14	GUATEMALA	3	6,706	99.970
15	NICARAGUA	1	6,707	99.985
16	COSTA RICA	1	6,708	100.000
17	PANAMA	0	6708	100.000
	TOTAL	**6,709**		

8 COUNTRIES %	9 COUNTRIES %	10 COUNTRIES %
98.53	99.27	99.57

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